Isolation and Identification of Chlorpyrifos Degrading Fungi from Pesticide Contaminated Agriculture Soil

Khushboo Minj [1], Jasmeet Kaur Sohal [2*]
[1] Research Scholar, School of Sciences, MATS University, Raipur, (C.G), India
[2] Associate Professor, School of Sciences, MATS University, Raipur, (C.G), India

* Corresponding Author: **Jasmeet Kaur Sohal**

Article Info

P-ISSN: 3051-3448
E-ISSN: 3051-3456
Volume: 07
Issue: 02
Received: 25-04-2026
Accepted: 27-05-2026
Published: 29-06-2026
Page No: 15-21

Abstract
Agricultural soils subjected to repeated pesticide application accumulate chlorpyrifos residues that persist for months to years, posing significant risks to soil health, non-target organisms, and groundwater quality. Bioremediation using indigenous fungal isolates offers a practical and ecologically sound approach to address this contamination. This study isolated and identified chlorpyrifos-degrading fungi from pesticide-contaminated agricultural soil in Okra village, Balrampur district, Chhattisgarh, India. Serial dilution plating on Potato Dextrose Agar yielded a fungal count of 4.2 × 10^4 CFU/g, from which three active degraders *Aspergillus niger*, *Trichoderma viride*, and *Aspergillus terreus* were confirmed by ITS region sequencing and NCBI BLAST analysis. Biodegradation was optimized across temperature, pH, nutrient concentration, aeration, and salt concentration. HPLC analysis over 30 days showed that *T. viride* achieved the highest degradation at 89.8%, followed by *A. niger* at 87.9% and *A. terreus* at 84.4%, compared to only 8.6% in the uninoculated control. First-order kinetic modeling confirmed half-lives of 8.5, 9.3, and 11.7 days respectively, representing up to a 34-fold reduction compared to abiotic degradation alone. These findings establish indigenous fungal isolates from Chhattisgarh as strong candidates for developing targeted bioremediation strategies against chlorpyrifos contamination in agricultural ecosystems.

DOI: https://doi.org/10.54660/JSFR.2026.7.2.15-21

Keywords: Chlorpyrifos, biodegradation, *Trichoderma viride*, *Aspergillus niger*, ITS sequencing, bioremediation, organophosphate

1. Introduction

The intensification of Indian agriculture following the Green Revolution of the 1960s brought a dramatic rise in the use of chemical pesticides and fertilizers to sustain crop yields. Chemical fertilizer consumption alone increased from one million tonnes in 1960 to 25.6 million tonnes by 2014-15, and pesticide use expanded proportionally (Government of India, 2016) [7]. While these inputs drove agricultural productivity, their unregulated and excessive application has led to persistent contamination of soil, water, and air, with documented consequences including soil fertility depletion, microbial population disruption, and severe public health outcomes including cancer, neurological disorders, and endocrine disruption (Zhang *et al.*, 2013; Abhilash and Singh, 2009) [17, 2].

Conventional physicochemical removal methods are costly and often incomplete, which has shifted research focus toward microbial bioremediation as a sustainable alternative. Fungal isolates remain comparatively underexplored despite their demonstrated degradation potential, largely because bacteria have gained more research interest due to their higher multiplication rate compared to fungi (El-Ghany & Masmali, 2016). Several studies have shown that fungal strains isolated directly from pesticide-contaminated soils exhibit high specificity and efficiency in chlorpyrifos breakdown, supporting the case for isolating indigenous fungal populations from contaminated agricultural soil rather than relying on generic laboratory strains.

Among the pesticides of greatest environmental concern are organophosphate insecticides, which account for a substantial share of global agricultural chemical use. Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is one of the most widely applied organophosphate insecticides, registered for use against a broad spectrum of pests including termites, mosquitoes, cutworms, cockroaches, and a range of agricultural insects across crops, lawns, and ornamental plants (Sunanda *et al*., 2016) [12]. As per data from the Directorate of Plant Protection, Quarantine and Storage, chlorpyrifos ranked as the ninth most consumed insecticide in India in 2009–10, reflecting its extensive use across the country. Its half-life in soil ranges from 10 to 120 days, and its primary metabolite, 3,5,6-trichloro-2-pyridinol (TCP), possesses antimicrobial properties that further inhibit the proliferation of chlorpyrifos-degrading microorganisms, compounding its environmental persistence (Singh and Walker, 2006) [13].

Prolonged environmental exposure to chlorpyrifos poses risks to non-target organisms including humans, with documented effects on the nervous system, internal organ function, and body weight in animal models (Sunanda *et al*., 2016) [12]. Conventional physicochemical remediation approaches for pesticide-contaminated soils are neither cost-effective nor environmentally sustainable, necessitating the development of biological alternatives.

Bioremediation, the use of microorganisms to degrade environmental contaminants, represents an ecologically appropriate and practically viable solution. Soil microorganisms, by virtue of their enzymatic versatility and metabolic adaptability, are capable of degrading pesticides into less toxic or non-toxic products, including complete mineralization to carbon dioxide and water (Singh and Walker, 2006; Cycoń and Piotrowska-Seget, 2019) [13, 6]. Fungi in particular possess a broad enzymatic arsenal including laccases, peroxidases, and esterases that enables them to degrade complex organic pollutants via both oxidative and hydrolytic pathways. Previous studies have reported fungal degradation of organophosphates and related compounds by *Aspergillus niger*, *Aspergillus flavus*, and *Trichoderma viride* (Gupta *et al*., 2012; Alrahman *et al*., 2013) [8, 3], confirming the potential of fungal isolates as bioremediation agents. Sites with a documented history of chlorpyrifos exposure are particularly productive sources for isolating adapted degraders, as long-term pesticide pressure selects for organisms that have developed tolerance or active degradation pathways.

Existing literature provides strong precedent for fungal-based chlorpyrifos remediation. Isolated mixed and pure fungal cultures from soil using an enrichment technique. The enriched mixed fungal cultures demonstrated the ability to biodegrade chlorpyrifos when cultivated in Czapek Dox medium, and *Acremonium* sp. was identified as an effective chlorpyrifos degrader. Similarly, a study on *Aspergillus niger* and *Trichoderma viride*, the same fungal genera investigated in the present study, found that *T. viride* exhibited greater chlorpyrifos-degrading efficiency, achieving 95.7% degradation compared with 72.3% by *A. niger* after 14 days of incubation. Furthermore, the toxic intermediate 3,5,6-trichloropyridinol (TCP) was not detected during the incubation period, indicating efficient degradation without accumulation of this harmful metabolite (Mukherjee and Gopal, 1996) [11]. Other researchers have similarly isolated chlorpyrifos-degrading fungi directly from contaminated soils demonstrated that chlorpyrifos biodegradation by a fungal consortium revealed strong potential for treating contaminated water and soil (Bhatt *et al*. 2021) [5]. While *A. niger*'s degradation capacity in pesticide-contaminated water. Parallel work on bacterial isolates from similar environments such as *Achromobacter xylosoxidans* and *Ochrobactrum* sp. strains that degraded 84.4% and 78.6% of chlorpyrifos within 10 days (Awad *et al.* 2011) [4] confirms that pesticide-contaminated agricultural soil is a reliable and productive source for isolating degrading microorganisms, reinforcing the methodological approach of this study.

Despite growing evidence for microbial chlorpyrifos degradation, studies focusing specifically on fungal isolates from indigenous Indian agricultural soils remain limited. Most reported isolates originate from laboratory collections or geographically distant sites, limiting their direct applicability to local soil conditions. The present study was therefore undertaken to isolate, identify, and characterize chlorpyrifos-degrading fungi from pesticide-contaminated agricultural soil in Balrampur district, Chhattisgarh, optimize biodegradation conditions, and quantify degradation efficiency using HPLC analysis. The findings aim to provide a foundation for developing indigenous, field-adapted fungal bioremediation strategies for chlorpyrifos-contaminated agricultural land in the region.

2. Materials and Methods

2.1. Soil Sample Collection and Processing

Soil samples were collected from agricultural fields in Okra village, Balrampur district, Chhattisgarh, India, with a documented history of repeated chlorpyrifos application. Samples were collected from 10-15 cm depth using sterile spatulas under aseptic conditions, pooled to form a representative composite sample, and transported to the laboratory in an ice box. Samples were stored at 4°C until processing. Prior to use, soil was passed through a ≤2 mm sieve to remove debris, and moisture content was maintained at 40% with sterile distilled water to support aerobic microbial activity (Gong *et al*., 2016) [9].

2.2. Isolation of Fungi

Serial dilutions of soil suspension (10^{-1} to 10^{-7}) were prepared in sterile distilled water. Appropriate dilutions were plated onto Potato Dextrose Agar (PDA; pH 5.6) by the pour plate method and incubated at 28 ± 1°C for 3-5 days. Morphologically distinct fungal colonies were selected and purified by repeated subculturing on fresh PDA plates. Pure cultures were maintained on PDA slants at 4°C for further studies.

2.3. Screening for Chlorpyrifos Degradation

Fungal isolates were screened for chlorpyrifos degradation using Minimal Salt Medium (MSM; KH_2PO_4 1.2 g, K_2HPO_4 4.8 g, NH_4NO_3 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $Ca(NO_3)_2 \cdot 4H_2O$ 0.04 g, $FeSO_4$ 0.001 g per liter, pH 7.0) supplemented with chlorpyrifos as the sole carbon source. Isolates were inoculated into MSM-chlorpyrifos flasks and incubated at 30°C on a rotary shaker at 120 rpm for 5–7 days. Biomass development and formation of clearance zones around colonies were used as indicators of degradation activity. Halo diameters were measured using a Vernier caliper and observations recorded in triplicate.

2.4. Morphological and Microscopic Characterization

Macroscopic characteristics of purified fungal isolates including colony color, texture, pigmentation, growth pattern, and sporulation were recorded after incubation on PDA at 28 ± 1°C for 3–5 days. Microscopic examination was performed using the Lactophenol Cotton Blue (LPCB) staining method. Fungal mycelium was mounted in LPCB solution (0.125 g cotton blue, 50 g phenol, 100 mL glycerol, 50 mL lactic acid in 50 mL distilled water) and examined at 40× magnification for spore morphology, hyphal structure, and other identifying features.

2.5. Molecular Identification by ITS Sequencing

Genomic DNA was extracted from 5-7 day old fungal mycelium using the CTAB method (Doyle and Doyle, 1987). DNA quality was assessed by 1% agarose gel electrophoresis and NanoDrop spectrophotometry (A260/A280 ratio 1.8–2.0). The ITS region was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White *et al*. (1990). PCR was performed in a 25 µL reaction volume under the following conditions:

Amplified products (~550–600 bp) were purified and subjected to Sanger sequencing. Consensus sequences were assembled using BioEdit v7.2 and identified by BLASTn analysis against the NCBI GenBank database. Isolates showing ≥97% sequence identity were identified to species level (Schoch *et al*., 2012) [15]. Sequences were deposited in GenBank and accession numbers obtained. A phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates in MEGA v11.0 (Tamura *et al*., 2021) [16].

2.6. Optimization of Biodegradation Conditions

The effect of temperature (20-45°C), pH (5-9), nutrient concentration (0.5-2.0% yeast extract and peptone), aeration (shaking vs. static), and salt concentration (NaCl and KCl, 0.5-2.5%) on chlorpyrifos degradation was evaluated individually in MSM-chlorpyrifos broth. Each variable was tested while maintaining all other parameters at their optimum. Samples were withdrawn at 24-hour intervals up to 120 hours and analyzed for residual chlorpyrifos and biomass.

2.7. HPLC Analysis

Residual chlorpyrifos was quantified at 7, 14, and 21-day intervals. Culture aliquots (20 mL) were centrifuged at 7,000 rpm for 10 minutes, and the supernatant was extracted with dichloromethane. The organic layer was dried, reconstituted in HPLC-grade acetonitrile, and filtered through a 0.22 µm nylon syringe filter prior to injection (Singh *et al*., 2004) [14]. Analysis was performed on a C18 reverse-phase column (250 × 4.6 mm, 5 µm) with acetonitrile : water (75:25 v/v, 1% phosphoric acid) as mobile phase at 0.8 mL/min, detection at 230 nm, and column temperature 40°C. A standard calibration curve was constructed from 10-100 mg/L chlorpyrifos standards. Degradation percentage was calculated as:

$$\text{Degradation (\%)} = [(C_0 - Ct) / C_0] \times 100$$

Degradation kinetics were modeled by first-order kinetics: $C(t) = C_0 \cdot e^{(-kt)}$, and half-life calculated as $t_{½} = 0.693/k$ (Singh *et al*., 2004) [14].

2.8. Statistical Analysis

All experiments were performed in triplicate and data expressed as mean ± SD. One-way ANOVA with Tukey's HSD post-hoc test was used to compare degradation efficiency among isolates. A p-value of <0.05 was considered statistically significant. Analysis was performed using GraphPad Prism.

3. Results

3.1. Soil Sample Collection and Microbial Count

Soil samples collected from Okra village, Balrampur district, Chhattisgarh exhibited characteristic odour and texture consistent with pesticide-exposed agricultural soil. Serial dilution plating on PDA yielded countable colonies at dilutions 10^{-3} to 10^{-5}, displayed with higher and lower dilutions producing either uncountable or too sparse growth respectively. The total fungal count was estimated at 4.2×10^4 CFU/g of soil. From 20 colonies observed on PDA plates, 14 morphologically distinct colonies were picked and 5 pure fungal cultures were obtained after repeated subculturing.

3.2. Screening for Chlorpyrifos Degradation

All five fungal isolates were inoculated onto MSM plates supplemented with chlorpyrifos as the sole carbon source. After incubation at 30°C for 5-7 days, three fungal isolates F1, F2, and F3 demonstrated visible growth, turbidity development, and formation of distinct clearance zones around their colonies, indicating active chlorpyrifos utilization. These three isolates were selected for all further studies.

3.3. Morphological Characterization of Fungal Isolates

3.3.1. Macroscopic Characteristics

Following incubation on PDA at 28 ± 1°C for 5 days, all three isolates produced morphologically distinct colonies. *Aspergillus niger* (F1) formed fast-growing colonies with a black to dark brown upper surface, pale yellow reverse, and powdery granular texture with abundant black sporulation, reaching 4.8 cm in diameter. *Trichoderma viride* (F2) was the fastest growing isolate, covering 7.2 cm within 5 days, with bright green cottony colonies, white reverse, and abundant green sporulation. *Aspergillus terreus* (F3) showed moderate growth (3.6 cm), with cinnamon brown velvety colonies, pale yellow-brown reverse, and abundant brown sporulation. All three isolates produced aerial mycelium as shown in **table 1**.

Table 1: Macroscopic Colony Morphology of Fungal Isolates

Characteristic	*A. niger* (F1)	*T. viride* (F2)	*A. terreus* (F3)
Colony colour (top)	Black to dark brown	Bright green	Cinnamon brown
Colony colour (reverse)	Pale yellow	White	Pale yellow-brown
Texture	Powdery granular	Cottony fluffy	Velvety powdery
Colony diameter (5 days)	4.8 cm	7.2 cm	3.6 cm
Sporulation	Abundant black	Abundant green	Abundant brown

3.3.2. Microscopic Characteristics by LPCB Staining

Microscopic examination following LPCB staining revealed distinct structural features for each isolate. *A. niger* (F1) showed characteristic black conidial heads with radiate arrangement, biseriate phialides, and large globose vesicles. *T. viride* (F2) displayed septate hyphae with distinctly branched conidiophores bearing clusters of green ellipsoidal conidia. *A. terreu*s (F3) showed compact columnar conidial heads with biseriate phialides and small globose conidia, consistent with its species description.

3.4. Molecular Identification by ITS Sequencing

3.4.1. DNA Extraction and PCR Amplification

Genomic DNA was successfully extracted from all three fungal isolates. NanoDrop spectrophotometry confirmed acceptable purity across all samples, with A260/A280 ratios ranging from 1.82 to 1.89. F2 (*T. viride*) yielded the highest DNA concentration (214.8 ng/µL), followed by F1 (*A. niger*) at 186.4 ng/µL and F3 (*A. terreus*) at 172.3 ng/µL. Agarose gel electrophoresis confirmed DNA integrity with clear, sharp bands and no smearing.

PCR amplification of the ITS region using the ITS1/ITS4 primer pair produced clear, specific bands for all three isolates within the expected range of 550–600 bp. F2 produced the largest amplicon at 586 bp, followed by F1 at 574 bp and F3 at 561 bp, confirming successful amplification suitable for sequencing.

3.4.2. BLAST Identification and Phylogenetic Analysis

BLASTn analysis of the consensus ITS sequences against the NCBI GenBank database confirmed species-level identification for all three isolates with high confidence (**Table 2**). F1 matched *Aspergillus niger* strain CBS 513.88 with 99.6% identity and 100% query coverage, F2 matched *Trichoderma viride* strain ATCC 28020 with 98.8% identity, and F3 matched *Aspergillus terreus* strain NIH 2624 with 99.1% identity. All E-values were 0.0, confirming statistically significant matches. The phylogenetic tree constructed using the Neighbor-Joining method with 1000 bootstrap replicates in MEGA v11.0 confirmed the clustering of each isolate with its respective reference species.

Table 2: NCBI BLAST Identification Results

Isolate	Amplicon (bp)	Closest NCBI Match	% Identity	Confirmed Species
F1	574	*A. niger* CBS 513.88	99.6%	*Aspergillus niger*
F2	586	*T. viride* ATCC 28020	98.8%	*Trichoderma viride*
F3	561	*A. terreus* NIH 2624	99.1%	*Aspergillus terreus*

3.4.3. Optimization of Biodegradation Conditions

Across all five parameters tested, the three fungal isolates showed consistent optimal ranges: 28°C temperature, pH 7.0, 1.0% nutrient concentration, shaking (aerated) conditions, and 0.5% NaCl. Under these optimal conditions, *T. viride* (F2) consistently performed best or near-best, achieving the highest degradation in temperature (86.2%), nutrient (84.2%), aeration (85.1%), and salt (85.2%) trials. *A. niger* (F1) led only in the pH trial (83.6%) and was otherwise second-best. *A. terreus* (F3) was consistently the weakest performer across all five parameters, though still showing substantial degradation (76-79% at optimal conditions).

Moving away from optimal conditions caused sharp drops in degradation across all parameters, but the severity varied. Aeration had the steepest effectswitching from shaking to static cut degradation by more than half (down to 35-41%). Temperature and pH extremes were similarly damaging, with degradation falling to 14-31% at high/low temperatures and to 12-18% under acidic pH. Nutrient and salt effects were directional but less extreme: too little nutrient (0.5%) or too much (2.0%) both reduced degradation, and salt tolerance was moderate, with degradation collapsing only at concentrations above 1.5-2.5% NaCl. Overall, the isolates need narrow, near-neutral, well-aerated, mesophilic conditions for peak chlorpyrifos breakdown and aeration is the single most sensitive variable in this dataset.

3.4.4. HPLC Analysis of Chlorpyrifos Degradation

Chlorpyrifos was detected at a retention time of 19.2 minutes at 230 nm. Chromatograms for all three fungal isolates and the uninoculated control were recorded at days 0, 7, 14, 21, and 30 (Figures 1–4). As in table 3 displayed the control retained 91.4% of the initial chlorpyrifos at day 30, confirming negligible abiotic degradation. In contrast, all three fungal isolates showed progressive and substantial reduction in chlorpyrifos concentration over time.

By day 30, *T. viride* (F2) achieved the highest degradation at 89.8 ± 0.95%, followed by *A. niger* (F1) at 87.9 ± 0.72% and *A. terreus* (F3) at 84.4 ± 0.95%. The rate of degradation was steepest between days 7 and 21, after which it slowed as residual substrate became limiting.

Table 3: Residual Chlorpyrifos Concentration Over Time (mg/L)

Isolate	Day 7 (%)	Day 14 (%)	Day 21 (%)	Day 30 (%)
F1 *A. niger*	47.7 ± 0.52	72.2 ± 0.63	83.2 ± 0.81	87.9 ± 0.72
F2 *T. viride*	53.2 ± 0.59	77.4 ± 0.66	85.1 ± 0.77	89.8 ± 0.95
F3 *A. terreus*	43.6 ± 0.97	65.8 ± 0.92	78.7 ± 1.03	84.4 ± 0.71
Control	3.8 ± 1.46	5.2 ± 0.05	6.9 ± 0.16	8.6 ± 0.24

Values are expressed as mean ± SD (n=3).

First-order kinetic modeling produced strong fits for all isolates ($R^2 > 0.99$). *T. viride* (F2) showed the highest rate constant ($k = 0.0816$ day^{-1}) with a half-life of 8.5 days, followed by *A. niger* (F1) at $k = 0.0742$ day^{-1} and half-life of 9.3 days, and *A. terreus* (F3) at $k = 0.0594$ day^{-1} and half-life of 11.7 days. The uninoculated control showed a half-life of 288.8 days, confirming that microbial activity, not abiotic factors, was responsible for chlorpyrifos breakdown with fungal degradation being 25 to 34 times faster than natural abiotic loss as shown in table 4.

Table 4: First Order Degradation Kinetics of Chlorpyrifos

Isolate	k (day^{-1})	Half-life (days)	R^2
F1 *A. niger*	0.0742	9.3	0.9934
F2 *T. viride*	0.0816	8.5	0.9948
F3 *A. terreus*	0.0594	11.7	0.9912
Control	0.0024	288.8	0.9842

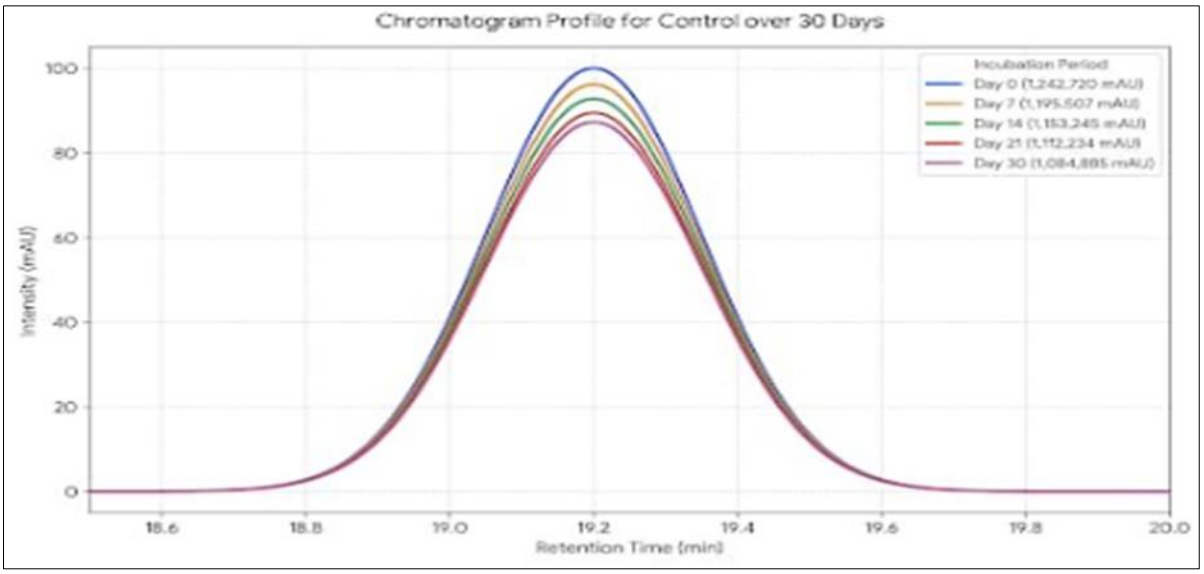

Fig 1: Chromatogram Observations for Chlorpyrifos of control (no fungi)

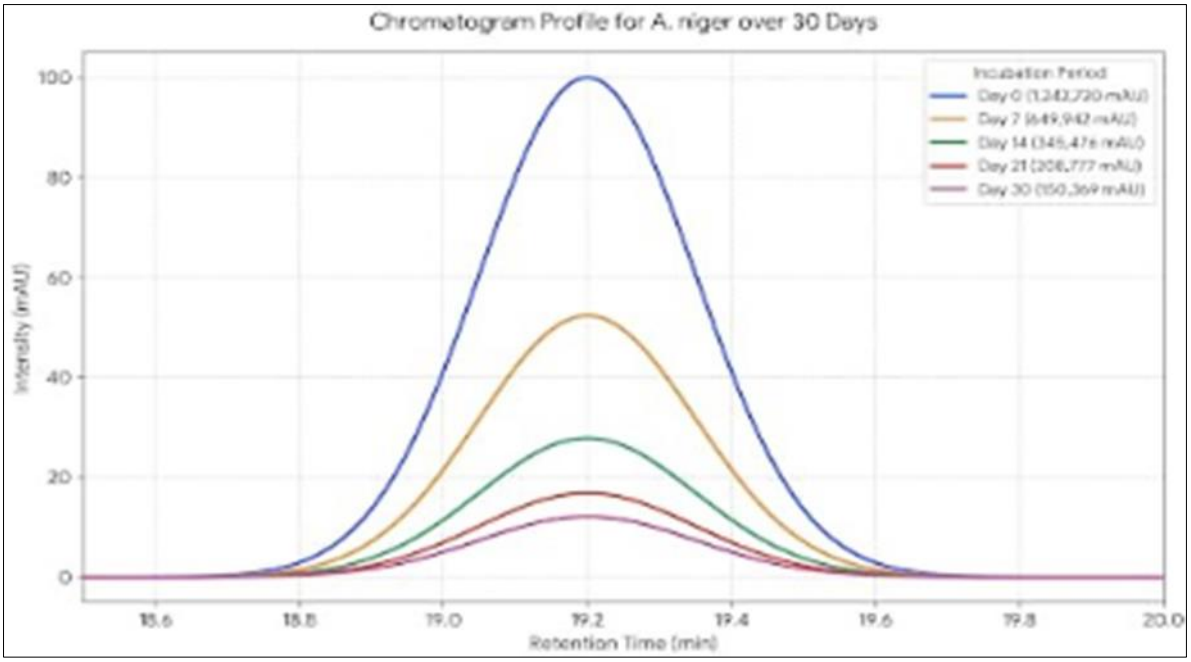

Fig 2: Chromatogram Observations for Chlorpyrifos of F1 *A. niger*

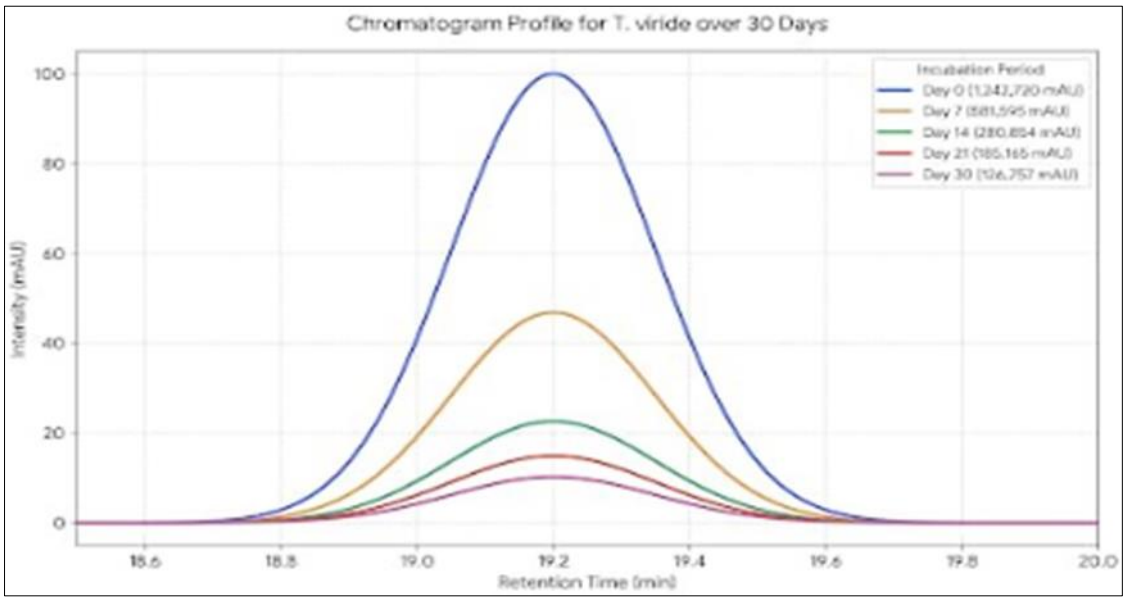

Fig 3: Chromatogram Observations for Chlorpyrifos of F2 *T. viride*

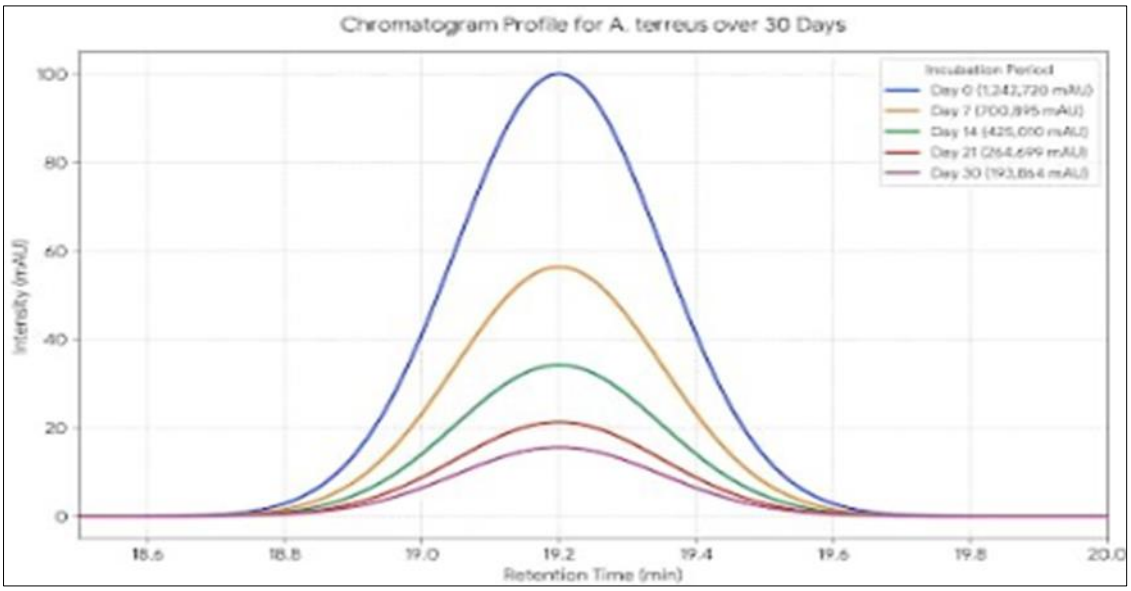

Fig 4: Chromatogram Observations for Chlorpyrifos of F3 *A. terreus*

4. Discussion

We have isolated a functionally active community from chlorpyrifos treated soil the counts of fungi reached a value of 4.2 x 10^4 CFU/g which matches published studies of various pesticide amended soil where cumulative exposure has led to development of adapted and degrading populations. The MSM screening system with only chlorpyrifos as a carbon source provided selection of organisms that possessed sufficient metabolic capability, the ability to transform (evidenced by development of zones of clearance and visible turbidity) later conirmed with HPLC.

Among these, *Trichoderma viride* (F2) yielded 89.8% degradation with a half-life period of 8.5 days and outdid the others significantly. It has been a general fact that *Trichoderma* species has enzymes such as laccases, peroxidases, and esterases which can effectively degrade varieties of pollutants such as organophosphates. *A. Niger* (F1) produced 87.9% degradation with a half-life period of 9.3 days, while *Aspergillus* species had been reported to degrade chlorpyrifos through oxidizing and hydrolyzing action. *A. terreus* (F3) gave 84.4% degradation and 11.7 days as half-life period and its performance seemed to be quite consistent but slow.

The similar optimum for the 3 fungal isolates at 28°C demonstrates the adaptation of many soil fungi to moderate environments, and the sharp drop above 35°C likely caused by denaturation of enzymes involved in the biotic metabolization of chlorpyrifos. Also, the neutral pH value optimum for all fungi active in degrading chlorpyrifos is an adaptive advantage, such that no artificial pH regime needed to be established or maintained in order for these fungi to perform well in field practices where the native pH of the farm soil rests around 7.0. The statistically significantly more efficient performance under shaking rather than static conditions confirms their O_2 dependence, also the magnitude of doubled degradation action under aeration is an engineering point worth noting. The value of 1.0% of water-soluble nutrients as optimal at which deterioration declines at higher concentrations shows that there's no advantage at all in adding too many nutrients to the tree: they are diverted to

other pathways rather than chlorpyrifos activation.

First-order kinetics provided an excellent fit for all isolates ($R^2 > 0.99$), confirming that degradation rate was proportional to residual substrate concentration. The uninoculated control retained 91.4% chlorpyrifos at day 30, corresponding to a half-life of 288.8 days, effectively ruling out abiotic contribution and confirming that the observed degradation was entirely microbial. The reduction in half-life to 8.5 days with T. viride represents approximately a 34-fold acceleration over abiotic loss alone.

The three fungal isolates characterized here were recovered directly from pesticide-exposed field soil in Chhattisgarh, meaning they are already adapted to regional environmental conditions. This increases their practical relevance for local bioremediation compared to laboratory stock isolates. Their co-occurrence with active bacterial degraders in the same soil further points toward the feasibility of consortium-based bioremediation systems, where bacterial and fungal degraders work simultaneously to achieve faster and more complete chlorpyrifos removal than any single isolate could accomplish alone. Future work should prioritize complete metabolite mapping using GC-MS or LC-MS, molecular characterization of the enzymes driving degradation, and soil microcosm experiments under ecologically realistic conditions before field-level application can be considered.

5. Conclusion

The present study isolated and identified three indigenous chlorpyrifos-degrading fungi—*Aspergillus niger*, *Trichoderma viride*, and *Aspergillus terreus*, from pesticide-contaminated agricultural soil of Balrampur district, Chhattisgarh. All isolates effectively utilized chlorpyrifos as the sole carbon source under aerobic conditions, with optimum degradation observed at 28°C, pH 7.0, and 1.0% nutrient concentration.

HPLC analysis over 30 days revealed that *T. viride* exhibited the highest degradation efficiency (89.8%; half-life 8.5 days), followed by *A. niger* (87.9%; half-life 9.3 days) and *A. terreus* (84.4%; half-life 11.7 days). In contrast, the uninoculated control showed only 8.6% degradation (half-life 288.8 days), confirming that chlorpyrifos removal was predominantly due to fungal activity. First-order kinetic modeling ($R^2 > 0.99$) accurately described the degradation process for all isolates.

Overall, *T. viride* and *A. niger* emerged as the most promising indigenous fungal candidates for the bioremediation of chlorpyrifos-contaminated agricultural soils. Their high degradation efficiency and adaptation to local environmental conditions highlight their potential for sustainable and eco-friendly bioremediation of organophosphate pesticide-contaminated sites.

References

1. Abd El-Ghany TM, Masmali IA. Fungal biodegradation of organophosphorus insecticides and their impact on soil microbial population. J Plant Pathol Microbiol. 2016;7:349. doi:10.4172/2157-7471.1000349.
2. Abhilash PC, Singh N. Pesticide use and application: An Indian scenario. J Hazard Mater. 2009;165(1-3):1-12.
3. Alrahman OA, *et al*. Butachlor degradation by *Pseudomonas* sp. But1 and *Pseudomonas* sp. But2 immobilized in polyurethane foam (PUF). Afr J Microbiol Res. 2013;7(12):1068-73.
4. Awad NS, Sabit HH, Abo-Aba SEM, Bayoumi RA. Isolation and molecular characterization of chlorpyrifos-degrading bacteria/fungi from agricultural wastewater. Afr J Microbiol Res. 2011.
5. Bhatt P, Huang Y, Zhan H, Chen S. Chlorpyrifos degradation using binary fungal strains isolated from industrial waste soil. Biologia. 2021;76:2901-16.
6. Cycoń M, Piotrowska-Seget Z. Pyrethroid-degrading microorganisms and their potential for bioremediation of contaminated soils. Front Microbiol. 2019;10:1255.
7. Government of India. Report of the Parliamentary Standing Committee on Agriculture. New Delhi: Ministry of Agriculture and Farmers Welfare; 2016.
8. Gupta M, *et al*. Fungal degradation of carbofuran by *Aspergillus* species. Pestic Biochem Physiol. 2012;102(1):26-31.
9. Gong T, Liu R, Zuo Z, Che Y, Yu H, Song C, *et al*. Metabolic engineering of *Pseudomonas putida* KT2440 for complete mineralization of methyl parathion and γ-hexachlorocyclohexane. ACS Synth Biol. 2016;5(5):434-42.
10. Kulshrestha G, Kumari A. Fungal degradation of chlorpyrifos by *Acremonium* sp. strain GFRC-1 isolated from a laboratory-enriched red agricultural soil. Biol Fertil Soils. 2011;47(2):219-25.
11. Mukherjee I, Gopal M. Degradation of chlorpyrifos by two soil fungi, *Aspergillus niger* and *Trichoderma viride*. Toxicol Environ Chem. 1996;57(1-4):145-51.
12. Sunanda M, Chandra Shekhar Rao J, Neelima P, Govinda Rao K, Simhachalam G. Effects of chlorpyrifos (an organophosphate insecticide) in fish. Int J Pharm Sci Rev Res. 2016;39(1):299-305.
13. Singh BK, Walker A. Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev. 2006;30(3):428-71.
14. Singh BK, Walker A, Morgan JAW, Wright DJ. Biodegradation of chlorpyrifos by *Enterobacter* strain B-14. Appl Environ Microbiol. 2004;70(8):4855-63.
15. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, *et al*. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci U S A. 2012;109(16):6241-6.
16. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-7.
17. Zhang M, Fang X, Zhou L, Su L, Zheng J, Jin M, *et al*. Pesticide poisoning in Zhejiang, China: a retrospective analysis of adult cases registered by occupational disease surveillance and reporting systems from 2006 to 2010. BMJ Open. 2013;3(11):e003510.

How to Cite This Article

Minj K, Sohal JK. Isolation and identification of chlorpyrifos degrading fungi from pesticide contaminated agriculture soil. Journal of Soil Future Research. 2026;7(2):15-21. doi:10.54660/JSFR.2026.7.2.15-21.