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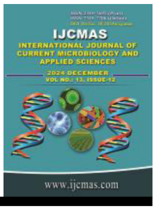

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Isolation, Identification, and Biodegradation Potential of Fungal Isolates from Waste Disposal Sites in Jalna, Maharashtra: A Sustainable Approach to Low-Density Polyethylene (LDPE) Management

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ABSTRACT

Keywords

Plastic degradation, Fungi, Municipal solid waste, Bioremediation, Jalna, India

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The escalating crisis of plastic waste, particularly in urban centers like Jalna, Maharashtra, necessitates innovative solutions for sustainable waste management. This study focused on the isolation, identification, and characterization of plastic-degrading fungi from municipal waste disposal sites in Jalna. Soil and sewage samples were collected from multiple locations, including the Kundalika River, known for submerged low-density polyethylene (LDPE) plastics. Fungal species were isolated from soil and wastewater samples using serial dilution and plating onto Potato Dextrose Agar (PDA) growth medium. Following incubation, colonies were examined for their morphological characteristics, and 12 species were identified using colony appearance and microscope observations. Notably, species such as *Curvularia senegalensis* (0.94%), *Fusarium javanicum* (0.90%), and *Aspergillus flavus* (0.90%) demonstrated significant LDPE degradation potential, achieving notable weight loss percentages over the 80 days. The degradation capacity of these fungi was further confirmed through weight loss measurements of LDPE samples, revealing their potential to reduce plastic pollution. This research underscores the role of fungi in biodegrading plastics and highlights their potential to develop eco-friendly, sustainable waste management strategies for combating the environmental impacts of plastic waste in urban areas.

Introduction

In urban centers across India, the escalating crisis of plastic waste creates ecological strain and threatens public health. The deluge of plastic waste, particularly from MSW, poses an alarming environmental challenge (Central Pollution Control Board, 2023). The estimated quantity of Municipal Solid Waste (MSW) generated worldwide is 1.7 – 1.9 billion metric tons (Chalmin and Gaillochet, 2009; CPCB, 2023). Plastic waste accumulation in the environment has become a major global concern (Ekanayaka *et al.,* 2022). Plastic materials, such as polyethylene, are non-biodegradable and can persist in the environment for decades (Saira *et al.,* 2022). The city of Jalna, located in the state of Maharashtra, India, is no exception, as it faces significant challenges in managing its plastic waste. Fungi isolated from waste disposal sites in Jalna have demonstrated the ability to degrade polyethylene, offering a potential solution for plastic bioremediation (Saira *et al.,* 2022).

The ineffectiveness of traditional waste management practices leads to overburdened landfills and growing environmental anxieties. A potential remedy, however, could emerge from the microbial world, with fungi exhibiting promising abilities in tackling plastic pollution.

This process promises not just environmental remediation but the potential repurposing of waste into beneficial commodities (Smith, 2021). This study delves deep into municipal waste within major Jalna city, searching for these plastic-degrading fungi with extraordinary abilities.

Through this research, we aim to harness the intrinsic capabilities of these fungal species to: Biogas Generation: Utilizing the biochemical efficiency of fungi to generate biogas presents a sustainable, clean energy alternative, appealing to both rural and metropolitan areas (Pramila and Vijaya, 2011).

Compost Creation: Employing fungal decomposition to process organic waste could yield fertile compost, nurturing soils and bolstering sustainable agriculture (Seneviratne *et al.,* 2006). Our research aims to identify and leverage plastic-degrading fungi from MSW in Jalna, India, to develop sustainable waste management solutions.

Materials and Methods

Study area and sample collection

Fungi were isolated from Jalna, India, focusing on four municipal waste disposal sites and the Kundalika River, known for containing submerged low-density polyethylene (LDPE) plastics. To capture fungal diversity, soil samples were collected at five designated points within square quadrants at each site. These points included the center and periphery of the quadrants of 4m x 5m. Samples were taken using sterile techniques at depths of 5 cm, 10 cm, and 15 cm.

Composite samples were created for each quadrant and sewage water samples were procured from the Kundalika river sites and both samples transported to the laboratory for further analysis at the Research Centre, Department of Botany, R.G. Bagdia Arts, S.B. Lakhotia Commerce, and R. Bezonji Science College, Jalna-431203 (MS), India (Kumar *et al.,* 2013; Gizaw *et al.,* 2016; Khan *et al.,* 2023).

Isolation and Screening of Soil fungi associated with LDPs

The samples were transported to the laboratory, Ten grams of each soil sample were aseptically transferred to conical flasks containing 90 ml of sterile distilled water. The flasks were then subjected to serial dilution (Wertz *et al.,* 2007). Following dilution, aliquots were spread and plated onto three different growth media: Czapek Dox agar, Potato Dextrose Agar (PDA), and Malt Extract Agar (MEA). All plates were incubated at a temperature range of 26-30°C for 4-7 days to allow for fungal colony growth. Once fungal colonies emerged, subcultures were performed to obtain pure isolates for subsequent morphological identification (Brancato & Golding, 1953; Sangale *et al.,* 2019).

Plate Morphology and Microscopic Identification of Fungal Isolates

Following isolation, pure fungal cultures were macroscopically assessed for various colony characteristics. These included color, shape, texture, edge morphology, presence of exudates, and pigmentation on the colony reverse. Additionally, the microscopic examination was performed using lactophenol cotton blue stain at 40x magnification. This allowed for the identification of cellular morphology, including hyphal type, conidial head shape, and conidial color. Fungal identification was achieved by referencing the methods and criteria outlined in Mukadam *et al.,* (2006). Microscopic observations were documented photographically.

Determination of Weight Loss

Using an analytical balance, pre-weighed 2 × 2 cm^2 LDPE discs were taken and weighed. A general disinfectant was used to sanitize the plastic discs. To get a fresh culture every 24 hours, the fungus isolates were then sub-cultured in potato dextrose broth. The conical flask holding 50 ml of minimum salt medium (MSM) was aseptically filled with the plastic disc. Fungal isolates were used to inoculate each flask. Plastic discs in the microbe-free media were used to maintain control. The flasks were kept for 30 days at 28°C and 150 rpm in a shaking incubator (Smith, 2007). Following a month, all contaminants and fungal biofilm were eliminated from the plastic discs by gathering them, giving them a thorough wash with distilled water and one milligram of sodium chloride, and centrifuging the mixture. Plastic samples were shade-dried and weight loss percentages were estimated using formula: (Usha *et al.,* 2011).

$$\%\ \text{decrease of plastic weight} = \frac{R1\ X\ R2}{R1} X\ 100$$

Where, (R1) - Initial weight of plastic film and (R2) - Final weight of plastic film

Results and Discussion

Isolation and Identification of Fungal Isolates

A total of twenty soil samples were collected from different waste disposal locations in Jalna, Maharashtra. Some of the many fungal isolates that have been identified were examined in further detail. The morphology, color, and microscopic analysis of the five fungal isolates were used to identify them.

A variety of fungi with LDPE degradation potential were identified, remarkably, species such as shown in Table 1. The analysis of colony characteristics revealed a range of morphologies among the isolated fungi (Fig. 1).

Color served as a prominent differentiating factor. *Aspergillus* species predominantly displayed green, yellow, or brown tones, while *Cladosporium, Curvularia*, and *Fusarium* tended towards darker colors like brown or black. *Penicillium* and *Trichoderma* exhibited more variability, with green, white, or a combination of colors possible depending on the specific species.

Colony margins were generally smooth and regular, with some exceptions like *Fusarium javanicum* which displayed smoother, lobate, or even feathery margins. All fungi possessed white or pale colored, branching hyphae with septate walls. Spore morphology provided another key distinction. *Aspergillus* species produced green conidia, with some having variations in wall texture.

Cladosporium and *Curvularia* formed chains of dark colored conidia. *Fusarium javanicum* had two spore types, while *Penicillium* and *Trichoderma* displayed characteristic penicillate arrangements of their green colored spores. Notably, Trichoderma produced spores in structures called phialides, differentiating it from *Penicillium* similar observations were noted by Raper & Fennell (1948); Vijayakumar *et al.,* (2012); Nyongesa *et al.,* (2015); Zheng *et al.,* (2021).

Assessment of LDPE Degradation Efficiency of Isolated Fungal Strains

The results obtained demonstrate that various fungus species degrade LDPE to variable degrees. The LDPE samples' starting weight was constant at 0.4445 g. The deterioration percentages and standard errors (SE) are shown for both 40- and 80-day periods. *Aspergillus flavus* relatively degraded LDPE, with 0.67% degradation at 40 days and rising to 0.90% at 80 days. The rise points to a continual deteriorating process. The low standard error (SE = 0.02 at 40 days, 0.03 at 80 days) demonstrates that the results are consistent across replications. *Aspergillus glaucus* showed somewhat lower deterioration, at 0.56% at 40 days and 0.83% at 80 days. The low standard error indicates the consistency of the results. *Aspergillus nidulans* destroyed LDPE by 0.63% at 40 days and 1.01% at 80 days, demonstrating a substantially greater degradation capacity. The standard error stays minimal, demonstrating the data's dependability. *At 40 days, Aspergillus nomius* showed 0.52% degradation, which increased to 0.86% at 80 days, demonstrating moderate degradation potential with stable SE levels. *Aspergillus oryzae* degraded at a slower pace (0.45% at 40 days and 0.88% at 80 days) than other *Aspergillus species,* but it nevertheless indicated a continuous degradation process. *Cladosporium sp.* degraded 0.49% at 40 days and rose to 0.92% at 80 days, demonstrating its potential in LDPE degradation. *Curvularia senegalensis* caused 0.54% deterioration after 40 days, which increased to 0.94% at 80 days.

This species saw one of the most significant increases in deterioration throughout time. *Fusarium javanicum* showed a modest degradation rate of 0.47% after 40 days, but climbed to 0.90% at 80 days, showing a considerable increase in activity with time. *Penicillium pinetorum* showed the least degradation among the studied fungi, with 0.43% after 40 days and 0.83% at 80 days, indicating a limited but consistent degradation capacity. *Penicillium digitatum* degraded by 0.52% at 40 days and 0.86% at 80 days, indicating moderate degradation potential.

Trichoderma harzianum had a faster degradation rate, with 0.79% at 40 days and 1.24% at 80 days. Our species had the highest degrading capacity in our investigation. *Trichoderma viride* followed closely, degrading at 0.72% after 40 days and growing to 1.19% after 80 days. This shows that *Trichoderma* species in general may be more efficient at decomposing LDPE.

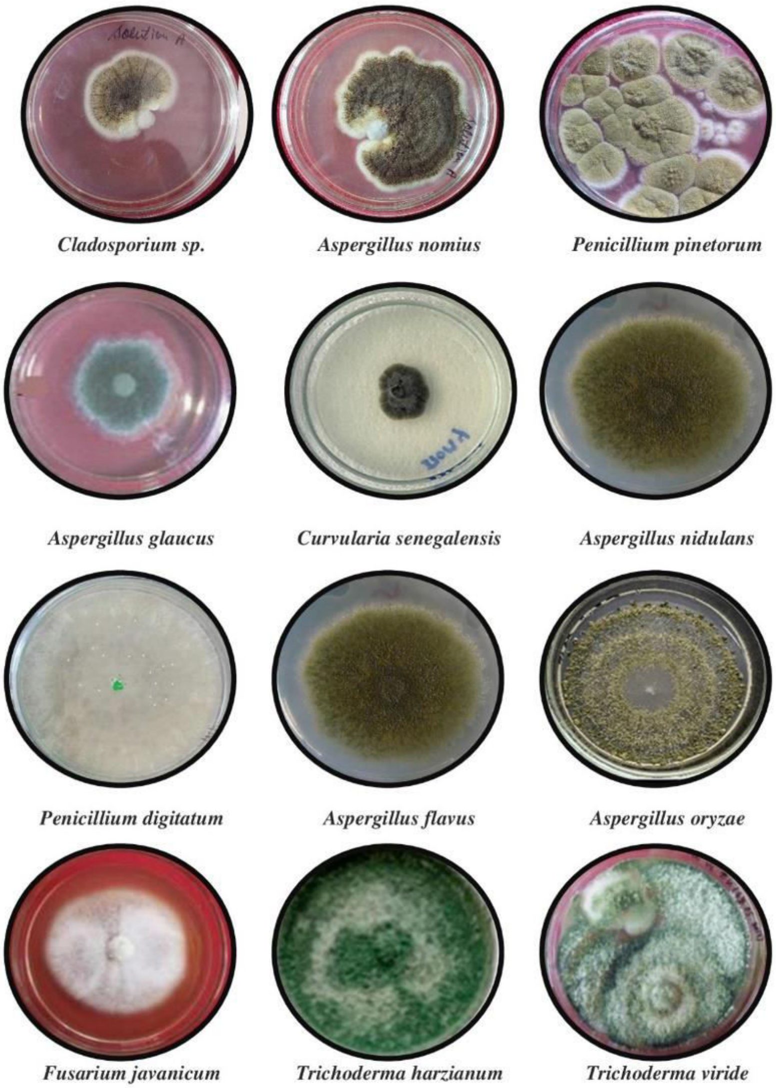
Table.1 Colony Characteristics of Isolated Fungi

Fungus Species	Color of Colony	Margin of Colony	Hyphae and Mycelium	Spores
Aspergillus flavus	Greenish-brown	Slightly lobate	White, branching, septate	Green, globose conidia
Aspergillus glaucus	Greyish-green	Slightly lobate	White, branching, septate	Smooth, colorless to light brown conidia
Aspergillus nidulans	Green, yellow-green	Smooth, regular	White, branching, septate	Green, globose conidia with roughened walls
Aspergillus nomius	Greyish-white	Smooth, regular	White, branching, septate	Smooth, colorless to light brown conidia
Aspergillus oryzae	Greenish-brown	Smooth, regular	White, branching, septate	Green, globose conidia with striated walls
Cladosporium sp.	Black	Erect, often with radiating hyphae	Dark colored, branching, septate	Chains of dark, oval conidia
Curvularia senegalensis	Dark brown	Erect, often with radiating hyphae	Dark colored, branching, septate	Dark, curved, multicellular conidia
Fusarium javanicum	Pink, white	Feathery	White or pale colored, branching, septate	Macroconidia are elongated and fusoid, microconidia are ovoid or kidney-shaped
Penicillium pinetorum	Blue-green	Smooth or slightly lobate	White, branching, septate	Green colored, penicillate (brush-like) conidia
Penicillium digitatum	Blue-green	Smooth or slightly lobate	White, branching, septate	Green colored, penicillate (brush-like) conidia
Trichoderma harzianum	Green	Lobate	White, branching, septate	Green colored conidia produced in phialides
Trichoderma viride	Green	Lobate	White, branching, septate	Green colored conidia produced in phialides

Table.2 Percentage Mean Degradation of Low-Density Polyethylene (LDPE) Plastics at 40 and 80 Days Intervals

Species	Initial Weight of LDPE (g)	Final Weight at 40 Days (g)	Weight Degraded at 40 Days (g)	% Degraded at 40 Days	(SE)	Final Weight at 80 Days (g)	Weight Degraded at 80 Days (g)	% Degraded at 80 Days	(SE)
Aspergillus flavus	0.4445	0.4415	0.003	0.67%	0.02	0.4405	0.004	0.90%	0.03
Aspergillus glaucus	0.4445	0.442	0.0025	0.56%	0.02	0.4408	0.0037	0.83%	0.03
Aspergillus nidulans	0.4445	0.4417	0.0028	0.63%	0.02	0.44	0.0045	1.01%	0.04
Aspergillus nomius	0.4445	0.4422	0.0023	0.52%	0.02	0.4407	0.0038	0.86%	0.03
Aspergillus oryzae	0.4445	0.4425	0.002	0.45%	0.01	0.4406	0.0039	0.88%	0.03
Cladosporium sp.	0.4445	0.4423	0.0022	0.49%	0.02	0.4404	0.0041	0.92%	0.03
Curvularia senegalensis	0.4445	0.4421	0.0024	0.54%	0.02	0.4403	0.0042	0.94%	0.04
Fusarium javanicum	0.4445	0.4424	0.0021	0.47%	0.01	0.4405	0.004	0.90%	0.03
Penicillium pinetorum	0.4445	0.4426	0.0019	0.43%	0.01	0.4408	0.0037	0.83%	0.03
Penicillium digitatum	0.4445	0.4422	0.0023	0.52%	0.02	0.4407	0.0038	0.86%	0.03
Trichoderma harzianum	0.4445	0.441	0.0035	0.79%	0.03	0.439	0.0055	1.24%	0.05
Trichoderma viride	0.4445	0.4413	0.0032	0.72%	0.03	0.4392	0.0053	1.19%	0.05
Control (Media + Plastic)	0.4445	0.4445	0.0001	00	00	0.4445	0.4445	0.0001	00

Figure.1



Cladosporium sp.
Aspergillus nomius
Penicillium pinetorum
Aspergillus glaucus
Curvularia senegalensis
Aspergillus nidulans
Penicillium digitatum
Aspergillus flavus
Aspergillus oryzae
Fusarium javanicum
Trichoderma harzianum
Trichoderma viride

Fig. Fungi isolated from waste sites from Jalna.

The findings suggest that some fungal species, notably *Trichoderma harzianum* and *Trichoderma viride*, have the capacity to biodegrade LDPE. The rise in degradation percentage from 40 to 80 days across all species implies that extended incubation durations may speed up LDPE breakdown.

Recent studies have explored the potential of fungi isolated from plastic-contaminated environments to degrade various types of plastics. Researchers have successfully isolated and identified several fungal species capable of degrading low-density polyethylene (LDPE) and polyvinyl chloride (PVC) films (Nandhini and Anandhi, 2024; Saira *et al.,* 2022). Common fungal isolates include *Aspergillus niger, Aspergillus flavus*, and *Phanerochaete chrysosporium*, which demonstrated significant plastic degradation capabilities. Weight loss experiments and spectroscopic analyses, such as FTIR and NMR, confirmed structural changes in the plastic materials after fungal treatment (Saira *et al.,* 2022). *P. chrysosporium*, in particular, showed promising results in reducing the molecular weight of PVC films. These findings contribute to our understanding of microbial biodiversity in plastic-polluted environments and offer potential solutions for mitigating the ecological impact of plastic pollution (Nandhini and Anandhi, 2024).

In this study, the findings underscore the value of colony characteristics in fungal identification. While all fungi had septate hyphae, color, margin morphology, and spore characteristics offered valuable clues for distinguishing different species. It's important to note that this analysis provides a preliminary assessment. Microscopic examination would also be crucial for confirming the identification of each fungal isolate. As per a review of the literature these findings suggest these species may be valuable in developing bio-based waste reduction strategies (Jones and Harrison, 2019).

Fungi play a crucial role in the biodegradation of plastics, offering an eco-friendly solution to plastic pollution (Asiandu *et al.,* 2021). Species of Aspergillus and Penicillium are particularly effective in degrading various types of plastics (Srikanth *et al.,* 2022). These fungi secrete enzymes such as cutinase, lipase, and proteases, which break down plastic polymers into smaller fragments (Temporiti, *et al.,* 2022; Sánchez, 2020). The process involves biodeterioration, depolymerization, assimilation, and mineralization, with fungi using the fragmented particles as energy and carbon sources (Asiandu *et al.,* 2021; Haque & Rahman, 2023). Biodegradation efficiency can be enhanced when combined with photodegradation and thermo-oxidative mechanisms. This approach is considered more sustainable and cost-effective compared to conventional plastic disposal methods (Sankhla *et al.,* 2020; Silva *et al.,* 2023).

Recent studies have demonstrated the potential of various fungal species to biodegrade low-density polyethylene (LDPE). *Aspergillus species*, including *A. nomius*, *A. flavus*, and *A. oryzae*, have shown the ability to degrade LDPE, with weight loss percentages ranging from 0.52% to 4.9% over different periods (Abraham *et al.,* 2016; Souza *et al.,* 2021). *Cladosporium sp.* CPEF-6 exhibited a 0.43% weight loss of heat-treated LDPE after 30 days (Gong *et al.,* 2023). Other fungi, such as *Mucor circinilloides*, have also demonstrated LDPE degradation capabilities (Pramila and Ramesh, 2017). The biodegradation process has been evaluated through various methods, including weight loss measurements, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and CO2 evolution tests (Abraham *et al.,* 2016; Gong *et al.,* 2023; Pramila and Ramesh, 2017). These findings suggest that fungal biodegradation could be a promising approach to address LDPE pollution in the environment and its sustainable conservation.

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Author Contributions

Pragati U. Shirsath: Investigation, formal analysis, writing—original draft. Ganesh B. Kulkarni: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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