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Biodegradation of Plastic Waste Using Various Fungal Isolates from Plastic-Polluted Sites in Meerut: Analysis of Polymers and Composites

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Abstract

By isolating microorganisms from a dumpsite containing low-density polyethylene (LDPE), the potential for microbial decomposition of plastic waste was studied. Four different strains of Aspergillus niger (fungus) were identified and used to biodegrade plastic waste. The isolated fungal strains included Aspergillus niger, Alternaria, A. fumigatus, A. flavus, and A. luchuensis. Predominant fungal strains, Aspergillus niger and Alternaria, were selected for polyethylene degradation under laboratory conditions. In a controlled shaker culture, the efficiency of the breakdown of low-density polyethylene (LDPE) and various polymer composites commonly used in plastic products was investigated over an 8-week period. Biodegradation was assessed through mean weight loss and Fourier-transform infrared (FTIR) spectroscopy. The study revealed a weight loss of approximately 14.3% to 28.57% after 8 weeks. Specifically, the total weight loss after 60 days was 14.3% for Alternaria sp. and 28.57% for Aspergillus niger. The findings suggest that both Alternaria sp. and Aspergillus niger possess the ability to degrade plastics, including various polymer composites. Notably, Aspergillus niger demonstrated superior efficiency in plastic degradation compared to Alternaria sp. This study highlights the potential of using fungal strains for the biodegradation of LDPE and composite polymers, contributing to more effective plastic waste management strategies.

Keywords: Polythene, biodegradable polymers, microorganisms, enzymes, heterotrophic fungi

INTRODUCTION

The majority of the industrial and technological advancements of the 19th and 20th centuries were fueled by fossil fuels, with plastics emerging as one of the most versatile synthetic materials ever developed (Raman et al., 2012) (27). Examples of various plastics include Polyethylene (PE), Polyethylene Terephthalate (PET), Nylons, Polypropylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR) (Strong, 2006) (36). However, discarded plastics, which are highly visible and rapidly accumulating in landfills, are resistant to biodegradation, leading to significant environmental pollution (Raman et al., 2012) (27).

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Previous research has demonstrated the biodegradation of polyethylene bags and plastic cups using microorganisms such as Aspergillus niger and Streptococcus lactis, with degradation efficiencies ranging from 12.25% to 12.5% (Priyanka et al., 2011) [1]. According to Gu et al. (2000) [2]. and Bhardwaj et al. (2012)(28), biodegradation involves the breakdown of materials by microorganisms through metabolic or enzymatic action (30). The extensive use of plastics has resulted in large quantities of plastic waste. Studies by Kubowicz et al. (2017) [3]. Varyan et al.

(2022)[4]. Zeenat et al. (2021) (6), and Di Mauro et al. (2021) (29) emphasize that biodegradation involves microbial organisms altering the structure of chemicals introduced into the environment. Considering the adverse effects of plastic waste, it is crucial to explore the microbial degradation of both natural and synthetic polymers, including composite materials used in modern applications (24). Biodegradable plastics and polymers offer promising avenues for innovative waste management strategies as they are designed to break down under environmental conditions or within municipal and industrial biological treatment facilities (Di Mauro et al., 2021) (29). The diversity of microorganisms capable of degrading these materials highlights their potential role in mitigating plastic pollution (Witt et al., 1997)(7).

Microorganisms exhibit remarkable adaptability to their environment, secreting both endoenzymes and exoenzymes that degrade substrates by cleaving molecular chains into smaller segments (Chandra & Rustgi, 1998)(8). Fungi that thrive under conditions of limited nutrition, variable pH, and moisture availability have demonstrated the ability to effectively decompose plastics to a useful extent (39). The application of biodegradable polymers is considered one of the most effective strategies for mitigating plastic pollution. These polymers are engineered to decompose rapidly due to the microorganisms' ability to break down both organic and inorganic compounds, such as lignin, starch, cellulose, and hemicelluloses (Kumar et al., 2013)(9). Research has confirmed that while microorganisms can degrade plastics in pure cultures, the potential of mixed microbial cultures for plastic degradation has not been thoroughly explored (Ogunbayo et al., 2019)(37).

Biodegradation of plastics often involves fungal colonization on the plastic surfaces, where fungi utilize the plastic as a food source. Ambient conditions, including pH and temperature, influence this process (23). The fungi secrete a variety of enzymes—such as cutinase, lipase, proteases, carboxylesterases, esterases, and lignocellulolytic enzymes—that facilitate the breakdown of plastics (Srikanth et al., 2022)(38). The use of biodegradable polymers, which are designed to degrade under environmental conditions, aligns with efforts to reduce plastic pollution effectively.

Fungi play a pivotal role in various biogeochemical processes and ecological niches. They contribute significantly to food web dynamics by breaking down resistant substrates, thus aiding the carbon cycle and nutrient recycling in terrestrial ecosystems (40). For example, mycorrhizal fungi enhance primary production in symbiotic relationships, while saprophytic fungi accelerate carbon and nitrogen cycles (Lindahl et al., 2007; Walder et al., 2012)(42). This diverse functional capability underscores the potential of leveraging fungal systems for enhanced plastic degradation and waste management strategies (43).

MATERIAL AND METHOD

Collection of Polythene Bags from Polluted Site of Modi Puram, Meerut

Polythene and soil samples (depth of approximately 5-10 cm) were collected from the garbage dumping site at Modipuram, Meerut (Uttar Pradesh).

Criteria of polymer selection

There are two types of Polythene: High Density Polythene (HDP) and Low -Density Polythene (LDP) here the polythene selected for biodegradation is LDP.

Isolation and identification of fungi

After being crushed and barely heated, a sample of 1 g of soil was diluted nine times in sterile distilled water. Serial diluents were aseptically added to several Petri plates, and then sterile media that had been cooled to 45 °C was added to the inoculums. It took several sub-culturing attempts to produce different colonies. Fungal isolates were subjected to microscopic and macroscopic examinations, including staining (with lactophenol cotton blue) for morphological features, and identification was made based on the characteristics (Adesemoye *et al*., 2006)(31). According to Singh and Gupta (2014), the biodegradation of plastic by microorganisms is often a very slow process and certain microbes are not able to break down all types of plastic. *Pseudomonas* sp., *Aspergillus glaucus* and *Pseudomonas* sp.

from mangrove soil degraded polythene and plastic with degradation efficiencies of 20.8%, 7.26%, 20.54%, and 8.16%, respectively (Kathiresan, 2003)(41), and *Bacillus cereus* from a plastic dumpsite degraded polythene with a degradation efficiency of 12.5% (Aswale and Ade, 2008)(44).

Isolation of Polythene degrading microorganisms

The polythene bags to be degraded were isolated from the polluted sites at a depth of 5-10cm and collected in a sterile plastic bag shown in Figure 1. After collection of plastic waste of Low Density Polythene it is washed with ethanol for removing dust and dirt particles and then weighed the plastic strips which are cut into 1cm diameter for studying its biodegradation by fungi (11).

Screening for polyethylene-degrading fungal isolates from soil samples

On potato dextrose agar (PDA) (Oxoid, Basingstoke, UK), standard dilution plating procedure was used for 72 hours at a temperature of 28 °C. By sub-culturing several colonies on recently made PDA medium, fungi were purified (Figure 2.1). Growing isolated colonies on plates using aseptically produced polypropylene Mineral Salt Medium (PMSM) allowed us to confirm the degrading ability of fungal isolates (19). All of the plates were incubated at 30 °C and growth was monitored for 3 to 7 days (Alshehrei, 2017) (32).

Characterization of heterotrophic fungi

After counting and estimating the total number of colonies, morphologically distinct colonies were selected and aseptically transferred to a sterile PDA agar substrate for further characterization. Selected strains of fungi are chosen for morphological characterization by microscopic method (Figure 2.2).

Screening and identification of polythene degrading fungi

The appropriate medium for the activity of enzymes contained in culture extracts of fungus was agar that had been supplemented with substrates such 1% starch, 1% gelatin, and 1% tween-80. Each substrate's agar amendment was sterilised individually at 15lbs for 15 minutes. In a sterile petri dish, 5–20 ml of sterilised substrate were added. The wells were sliced aseptically to load the culture filtrates of isolated fungus after the plates had been surface dried overnight. The culture filtrate-loaded plates were incubated for 3–4 hours at 37 degrees. Opacity was seen around the well surface after incubation, indicating a successful outcome for the substrates.

Microbial degradation of plastics

Reduced Density One-cm-diameter polythene strips that had already been weighted were aseptically transferred to conical flasks that contained 100ml of Rose Bengal broth medium, and each flask was then individually infected with the chosen fungal strains.

Figure 1. Plastic waste collection of dumping site and collection of soil from plastic dumping site.

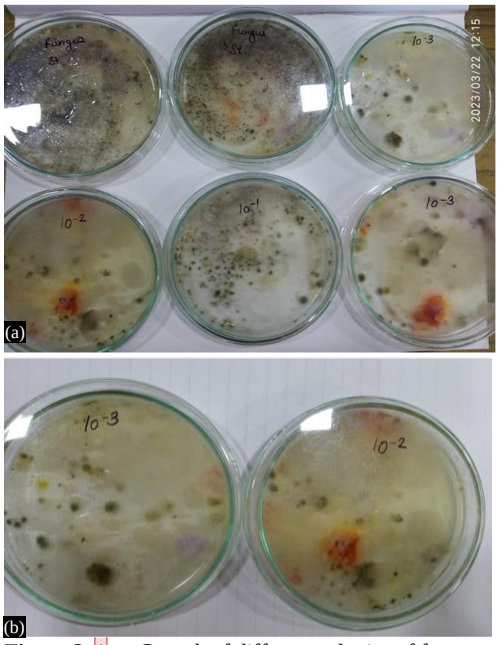

Figure 2. 1: a. Growth of different colonies of fungus at different dilutions

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Low-density polyethylene strips in the microbe-free media were used to maintain control. For each treatment, five flasks were kept and placed in a shaker. The polyethene strips were collected, cleaned with distil water and ethanol, and weighed to determine the total weight loss after two months of shaking. The weight loss of the polythene strips was computed using the data gathered. There was further picture analysis done.

Measurement of residual substrate

After exposure to the fungus, the test tubes holding the plastic samples were removed and carefully cleaned with ethanol shown in Figure 3 and 4. The strips were then dried overnight at 60 degrees Celsius, and the % weight loss was calculated using the formula below (Table 2).

$$\text{Weight Loss (\%)} = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

Control Flask

- Strain 1- *Alternaria*
- Strain 2-*Aspergillus niger*
- Strain 3-*Aspergillus fumigatus*
- Strain 4-*Aspergillus luchuensis*

RESULTS

Fungi isolated from polluted site were identified as– *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus luchuensis*, *Alternaria* and *Aspergillus flavus*. Four prominent fungi - *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus luchuensis* and *Alternaria* were selected for further studies.

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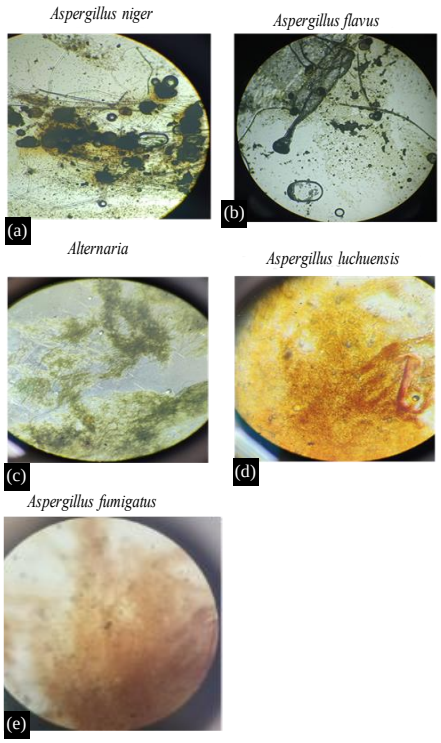

Figure 2. 2: Microscopic vision of different strains of fungal colonies.

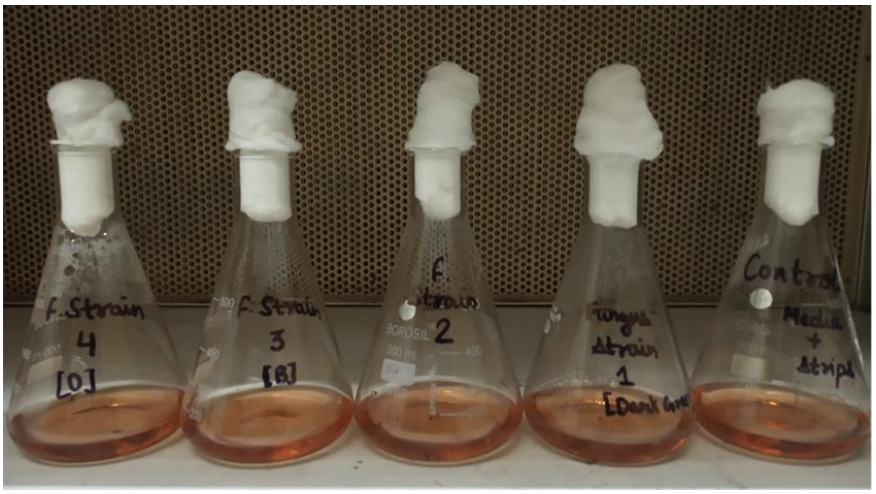

Figure 3. Each flask containing different fungal strain with plastic strips and controlled flask with plastic strip without microbial culture.

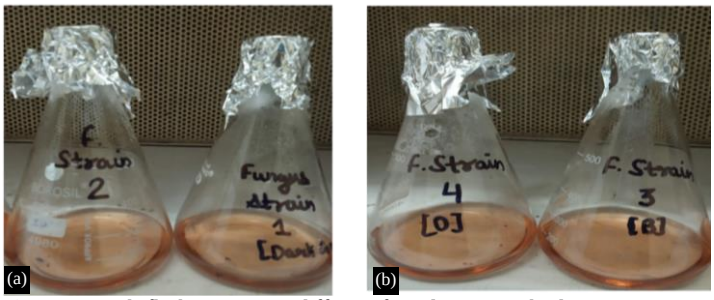

Figure 4. Each flask containing different fungal strain with plastic strips: a. strain 1- Alternaria and strain 2- Aspergillus niger and b. strain3- Aspergillus fumigatus and strain 4 - Aspergillus luchuensis.

The weight loss studies shows the physical changes and FTIR of the polythene shows the chemical changes that occurred due to the liberation of carbonyl compounds, compounds of lower molecular weights, after treating them with different strains of fungi.

The FTIR analysis of the degraded LDPE films exhibited variations in the polymer backbone. Carboxylic acids, alkenes, alkanes, alcohols, ketones and esters were some of the end products noticed after degradation of polythene with different strains of fungi.

The surface functional group alterations of the plastic film made from Low Density Polyethene plastic waste were supported by the results of FTIR spectra (Table 1, Figure. 5.1–5.4). In the current investigation, the treated plastic film with fungal biomass and the control plastic film with no fungi revealed only 5 peaks of control (2915.93cm^{-1}, 1462.90cm^{-1}, 1035.29cm^{-1}, 718.95cm^{-1}) and 14 peaks (2916.46cm^{-1}, 2848.19cm^{-1}, 1500cm^{-1},719.03cm^{-1}, 500cm^{-1}, 2916.29cm^{-1}, 2848.77cm^{-1}, 1463.09cm^{-1}, 1030.95cm^{-1}, 719.01cm^{-1}, 2800cm^{-1}, 2000cm^{-1}, 1000cm^{-1}, 740cm^{-1}, 500cm^{-1}) of treated plastic film with fungal biomass respectively. The number of comparable or distinct functional groups will undoubtedly increase as the number of peaks in the treated plastic films increases. The more functional groups there are, the more likely it is that the metal molecule will bind to newly developed groups and boost the usefulness of the biomass. Following the loading of the metal, certain peaks disappeared along with their corresponding groups from the spectra, and other peaks developed. Strong groups were present in the unloaded biomass, including the ring deformation of phenyl, CH_3 asymmetric deformation, stretching C-N, deformation N-H, deformation C-H, and Adenine vibrations in DNA. These groups disappeared from the spectra after the metal was loaded, and they were likely involved in the uptake of metals. The proportion of b-sheet secondary structures increased along with the quantity, exposing more functional groups to the environment stretching vibrations of CH_2 and CH_3, which can have a substantial role, stretching C-N, cholesterol, phospholipids, and creatine.

The peaks observed in liquid sample which is treated with plastic film and fungal biomass revealed 2 peaks of control sample (3346.66cm^{-1}, 1635.96cm^{-1}) and 8 peaks (3350.66cm^{-1}, 1635.96cm^{-1}, 3350.68cm^{-1}, 1635.20cm^{-1}, 3348.31cm^{-1}, 1635.23cm^{-1}, 3348.31cm^{-1}, 1635.23cm^{-1}, 3346.66cm^{-1} and 1635.95cm^{-1})(fig.-5.5, 5.6, 5.7, 5.8 and 5.9) the peaks observed of control media at wavenumber- 2915.93cm^{-1}, 1462.90cm^{-1}, 1035.29cm^{-1} and 718.95cm^{-1} (fig.-5.1)which shows the presence of Stretching vibrations of CH2 & CH3 of phospholipids, cholesterol and creatine (Huleihel *et al*.,2002)(10), Stretching C-O ribose (Dovbeshko *et al*.,1997)(17), n(CH_2OH), n(CO) stretching coupled with C-O bending Glycogen (Wood *et al.*, 1996) and Out-of-plane bending vibrations (Schulz, H. and Baranska, M. (2007) and the peaks observed of treated fungal biomass plastic film and 14 peaks at wavenumber- 2916.46cm^{-1}, 2848.19cm^{-1}, 1500cm^{-1}, 719.03cm^{-1}, 500cm^{-1}, 2916.29cm^{-1}, 2848.77cm^{-1}, 1463.09cm^{-1}, 1030.95cm^{-1}, 719.01cm^{-1}, 2800cm^{-1}, 2000cm^{-1}, 1000cm^{-1}, 740cm^{-1}, 500cm^{-1} (fig.- 5.2-5.4)(13) in comparison to control sample some peaks are diasppered and new peaks are observed due to the presence of fungal biomass and breakdown of plastic films and interaction between them. (fig.-5.5) which shows Stretching N-H asymmetric (Dovbeshko *et.al*.,1997)(17).and 8 peaks (3350.66cm^{-1},

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1635.96cm^{-1}, 3350.68cm^{-1}, 1635.20cm^{-1}, 3348.31cm^{-1}, 1635.23cm^{-1}, 3348.31cm^{-1}, 1635.23cm^{-1}, 3346.66cm^{-1} and 1635.95cm^{-1}) (fig.-5.5, 5.6, 5.7, 5.8 and 5.9).

Table 1. FTIR absorption peaks and their respective chemical functional group

Peaks	Assignment	Refrences
2915.93- 2916 cm^{-1}	Cholesterol, phospholipids and creatine (higher in normal tissues). Stretching vibrations of CH_2 & CH_3 of phospholipids, cholesterol and creatine.	Huleihel *et al*.,2002(10) Huleihel *et al*.,2002(10)
1462.90cm^{-1}	Paraffin	Dukor, R.K. (2002)
1035.29cm^{-1}	Skeletal trans conformation (CC) of DNA n(CC) skeletal cis conformation, n(CH_2OH), n(CO) stretching coupled with C-O bending Glycogenn (CO), n(CC), n(CCO), (polysaccharidescellulose)	Huleihel *et al*., 2002 (10) Fujioka *et al*., 2004 (12) Wood *et al.*, 1998 Shetty *et al*.,2006 (14)
700-1000cm^{-1}	Out-of-plane bending vibrations	Schulz, H. and Baranska, M. (2007) (15)
2916.46cm^{-1}	Cholesterol, phospholipids and creatine (higher in normal tissues). Stretching vibrations of CH_2 & CH_3 of phospholipids, cholesterol and creatine.	Huleihel *et al*., 2002 (10)
2848.19cm^{-1}	Cholesterol, phospholipids, and creatine (higher in normal tissues) Stretching vibrations of CH_2 & CH_3 of phospholipids, cholesterol, and creatine	Huleihel *et al*., 2002 (10) Huleihel *et al*., 2002 (10)
1500cm^{-1}	In-plane CH bending vibration from the phenyl rings	Schulz, H. and Baranska, M. (2007)(15)
2916.29cm^{-1}	Cholesterol, phospholipids and creatine (higher in normal tissues). Stretching vibrations of CH_2 & CH_3 of phospholipids, cholesterol and creatine.	Huleihel *et al*., 2002 (10)
2848.77cm^{-1}	Cholesterol, phospholipids, and creatine (higher in normal tissues) Stretching vibrations of CH_2 & CH_3 of phospholipids, cholesterol, and creatine	Huleihel *et al*., 2002 (10)
1463.09-1464cm^{-1}	Stretching C-O ribose	Dovbeshko *et al*.,2000 (17)
1030.95cm^{-1}	Glycogen vibration CH_2OH vibrationns C-O	Wang *et al*., 1997 (18) Gazi *et al*.,2003 Wang *et al*.,1997 (18) Paluszkiewicz *et al*.,2001 (20)
2800cm^{-1}	Stretching N-H (NH_3 þ)	Dovbeshko *et al*.,*2000 (17)*
1000- 650cm^{-1}	Porphyrin ring of heme proteins	Alfano *et al*.,1987 (33)
1000-350cm^{-1}	Region of the phosphate vibration Carbohydrate residues attached to collagen and amide III vibration (in collagen)	Dovbeshko *et al*.,2000 (17) Fabian *et al*.,1995 (21)
3343-3346.66cm^{-1}	Stretching N-H asymmetric	Dovbeshko *et.al*.,2000 (17)
1635.98-1636cm^{-1}	b-sheet structure of amide I. Proportion of b-sheet secondary structures (shoulder)	Eckel *et al*.,2001 (22) Fabian *et al*.,1995(21)
3350.68cm^{-1}	O-H, N-H, C-H	Dovbeshko *et al*.,2002 (16)

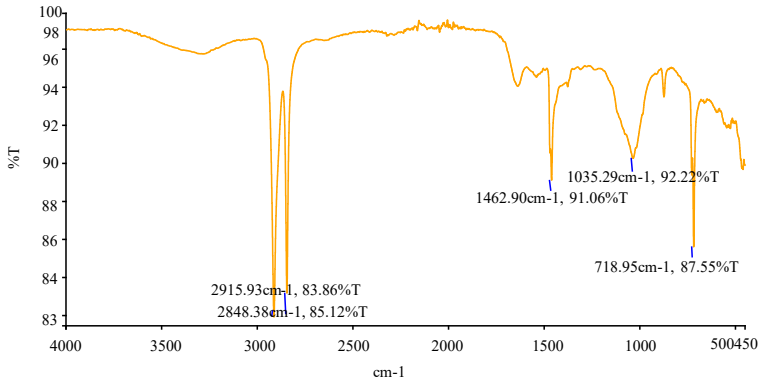

Figure 5. 1: FTIR analysis of LDPE control.

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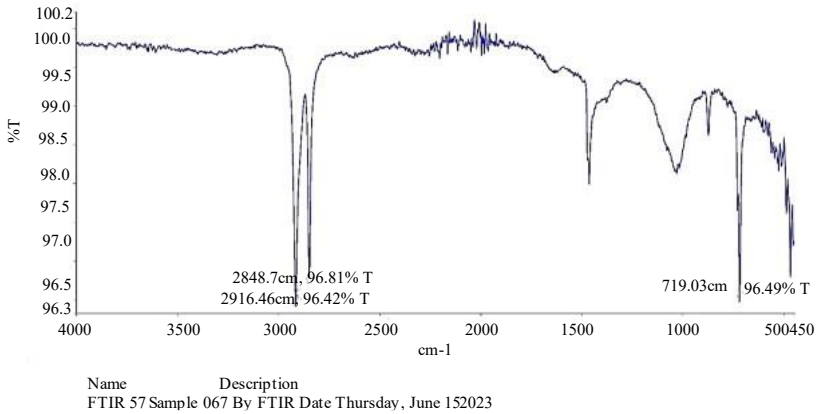

Figure 5. 2: FTIR Analysis of LDPE treated with *Aspergillus niger*.

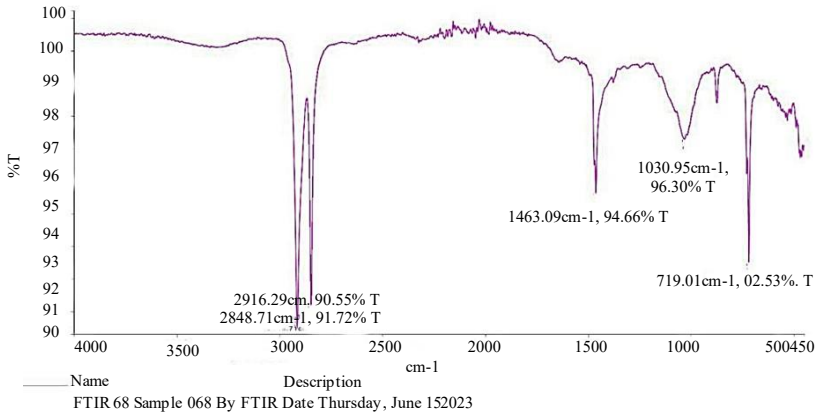

Figure 5. 3: FTIR Analysis of LDPE treated with *Aspergillus fumigatus*.

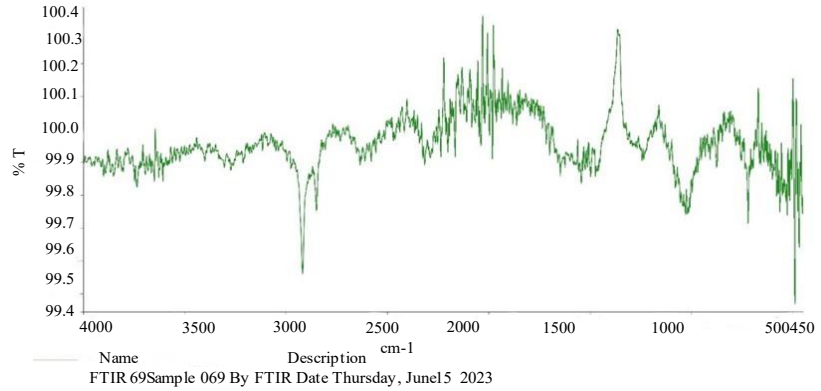

Figure 5. 4: FTIR Analysis of LDPE treated with *Aspergillus luchuensis*.

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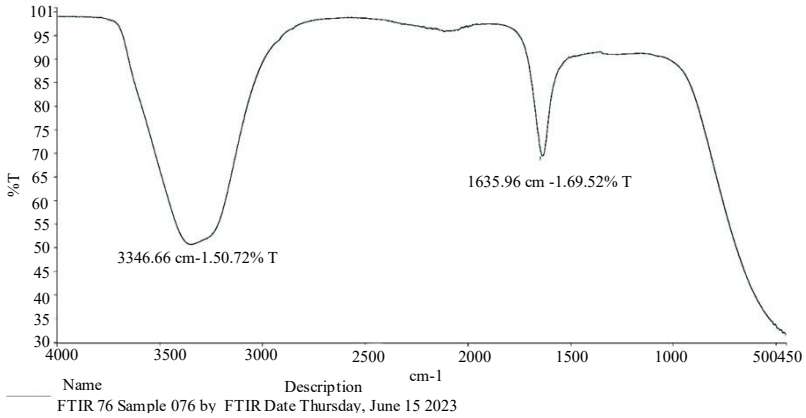

Figure 5. 5: FTIR Analysis of control liquid media with LDPE.

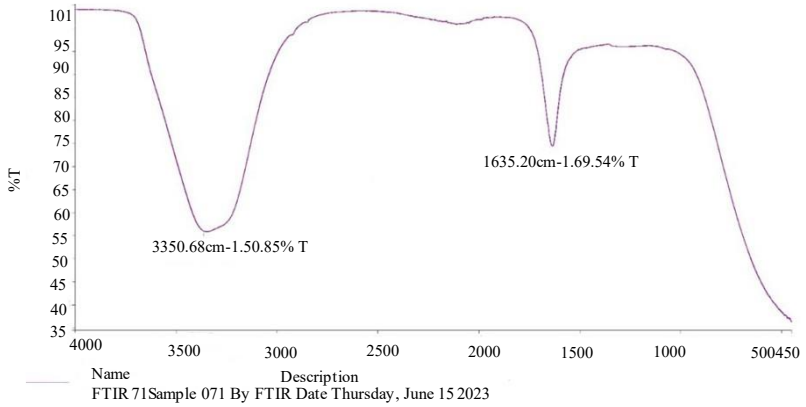

Figure 5. 6: FTIR Analysis of Liquid media containing biomass of Alternaria and LDPE strips.

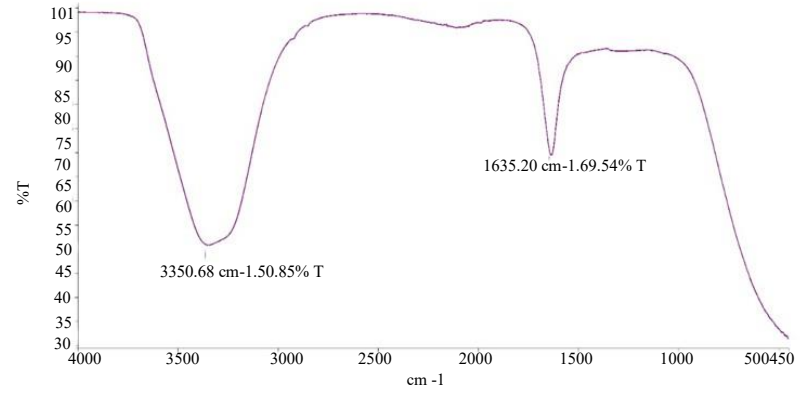

Figure 5. 7: FTIR Analysis of Liquid media containing biomass of *Aspergillus niger* and LDPE strips.

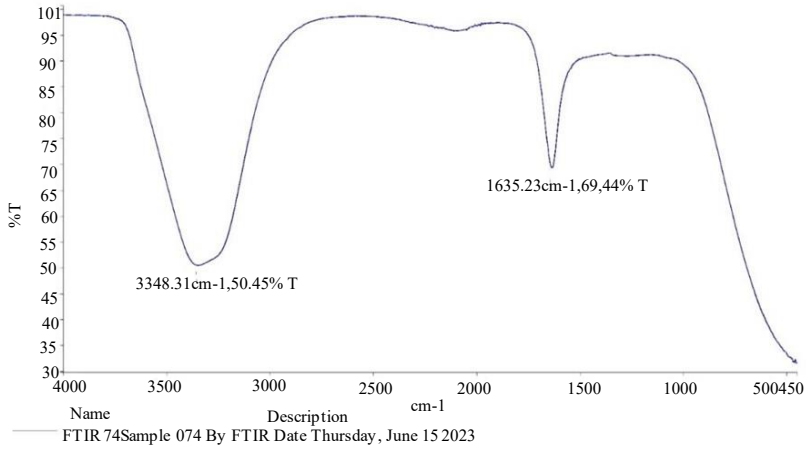

Figure 5. 8: FTIR Analysis of Liquid media containing biomass of *Aspergillus fumigatus* and LDPE strips.

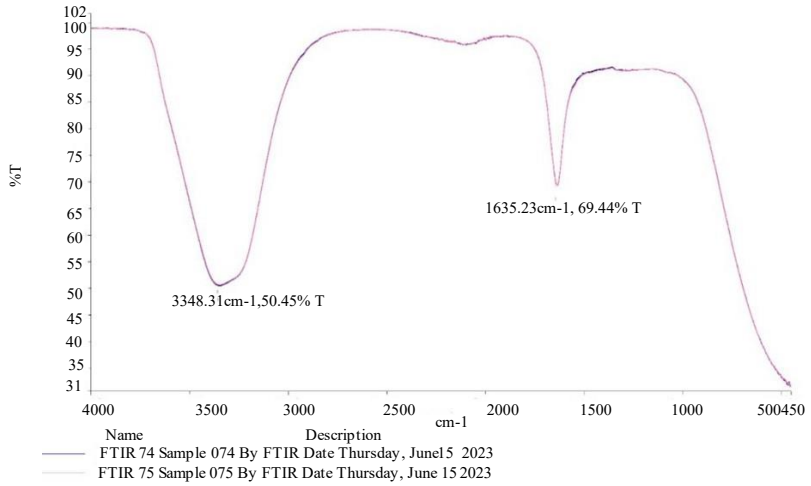

Figure 5. 9: FTIR Analysis of Liquid media containing biomass of *Aspergillus luchuensis* and LDPE strips.

DISCUSSION

In natural ecosystems, microorganisms play a crucial role in the biological degradation of materials, including manmade polymers. The most widely used synthetic plastics are made of high- and low-density polyethylenes, but because they degrade slowly in natural settings, they pose a severe threat to the environment. In this context, non-degradable synthetic polymer biodegradation utilising efficient microorganisms is gaining popularity. (Gu, 2003; Lee *et al*., 1991). There is no report on polythene degradation by *Alternaria* so far. This is the first experimental report of low-density polythene (LDPE) degradation under laboratory conditions by showing effective ability of *Alternaria*. The potency of degradation by *Alternaria* is 14.3% and of *Aspergillus niger* is 28.57%.

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Table 2. Percentage of Plastic biodegradation in 2 months.

Fungal Strains	Pre- weighed LDPE	Weighed after 2 months incubation in microbial culture	Percentage of plastic degradation by fungal strains
Control + LDPE Strips	7mg	7mg	0%
Alternaria	7mg	6mg	14.3%
Aspergillus niger	7mg	5mg	28.57%
Aspergillus fumigatus	7mg	7mg	0%
Aspergillus luchuensis	7mg	7mg	0%

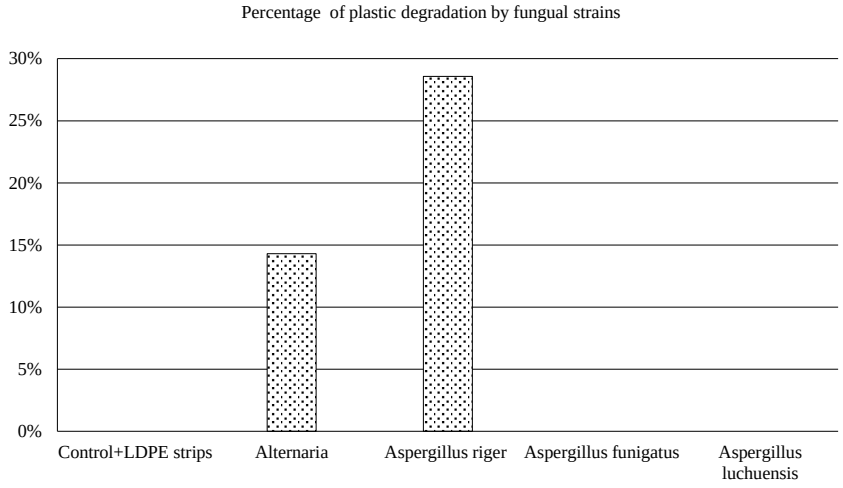

Figure 6. Graph showing percentage degradation of plastic in 2 months.

In addition to abiotic soil variables as moisture, heat, and temperature, the presence of fungus in the soil at polluted sites has caused degradation of the polythene bags (Anonymous, 1999) (34). The specific method of deterioration is unknown, and the plastic material's surface has changed from being smooth to being rough and cracked. Extracellular and intracellular depolymerases are at least two kinds of enzymes that are actively involved in the biological breakdown of polymers throughout the depolymerization process (Gu *et al*., 2000) (35).

Exo-enzymes from microorganisms break down complex polymers during degradation, producing smaller molecules with short chains, such as oligomers, dimers, and monomers, which can then be used as carbon and energy sources by the microbes due to their semi-permeable outer membranes (Frazer, 1994(25); Hamilton *et al*., 1995) (26). Therefore, more research into the role of microbial enzymes or organic acids in the breakdown of polythene and plastic will pave the way for the development of technologies to destroy these environmentally harmful plastic materials. According to earlier research on *Aspergillus niger*, plastic may degrade at a rate of 5.8% each month. However, in this investigation, I discovered that plastic trash can degrade at a rate of 28.57% in around two months.

CONCLUSIONS

Fungal strains -*Aspergillus niger, Alternaria, fumigatus and A. luchuensis* were selected for polythene degradation under laboratory conditions. Their effectiveness on the degradation of commercial polythene carry bags of low- density polyethylene was studied over a period of 8 weeks. Biodegradation was measured in terms of mean weight loss, which was nearly 14.3- 28.57%.

Accumulation of plastic waste is a serious environmental issue which is to be treated properly so that it can be less toxic to living beings and environments. Biodegradation of plastic can be viewed as one of the strategic studies to overcome this problem. In this study, the biodegradation of plastic using *Aspergillus niger* (fungus) and *Alternaria* isolated from soil samples collected from plastic polluted site of Modipuram, Meerut (Uttar Pradesh) were evaluated. The weight loss of each experiment is monitored and evaluated over a period of 60 days was-28.57% and 14.3% (Shown in Figure 6). It can be inferred that *Aspergillus niger* and *Alternaria* have the ability to degrade plastics. From the results obtained it can be inferred that *Aspergillus niger* have the better ability to degrade plastics than *Alternaria* under the conditions used for experiment.

Further analysis was done by FTIR Analysis which show degradation of plastic strips and breakdown of compounds like – carboxylic acid, alcohol, ketone and esters (Shown in Figure 5.1- 5.9).

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