



ISOLATION AND IDENTIFICATION OF FUNGAL FLORA ON DEGRADING POLYTHENE HEAPS AROUND WATER BODIES OF JHANSI

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Polythene is an important part of daily life, that's why demand of polythene production is increasing worldwide each year but its final non-treated waste is making earth polluted. Polythene is becoming major threaten element for environment, seems lack of awareness and poor waste management in last two decade. Fungi are ubiquitously found as reported from in each and every habitat, even can survive in adverse environment. Biodegradation is a best way, if possible, to overcome this problem. Still fungal flora of decaying polythene waste is not much worked out. This piece of work was conducted in order to find out a microbial consortium for the biodegradation of polythene. Samples were collected from various water bodies of Jhansi and screened for their fungal flora. In present study 10 fungal strains were isolated from different natural decaying sites.

Keywords: Biodegradation; Fungi; Land pollution; LDPE; Persistent organic pollutants; Polythene.

Introduction

Polythene is the most versatile synthetic manmade substance created out of the fossil fuel and other organic material extracted from coal, oil and natural gas¹. Polythene are nonmetallic moldable compounds that can be pushed into almost any desirable shape and then retain in that shape². As the consumption is growing, parallel waste management by chemical and physical methods becoming a challenging task as it produces persistent organic pollutants (POP's) known as furans and dioxins³. To get rid of such a menace, commonly landfill and burning has done that is the core origin of pollution. On the other hand, Plastic

sheets or bags do not allow water and air to go into earth which causes infertility of soil, preventing degradation of other normal substances, depletion of underground water source, danger for animal life and the main cause of blocked drains⁴. Promiscuous and frequently deliberate release of plastics is responsible for escalating environmental pollution. To remuneration this environmental damage, there is an urgent need of potential method.

Biodegradation executed by the microorganisms has environmental-friendly benefit and this method is less expensive and alternative of decaying (Physical and chemical changes) organic pollutant⁵.

Among all biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of polythene and rich microbial flora easily originates near water bodies because of its friendly environmental condition. Sometime submerged polythene found with heavy fungal growth, slime and soft textured, tearing easily with brittle nature. Fungi are vigorously found, easily grown and a diverse group of organisms belonging to saprophytic fungi. Fungi are evolved to adapt almost every environment, specially marine and freshwater source. This property drives fungi to grown on polythene even in adverse environment. A verity of heterotrophic fungi associated with polythene, is reported decaying sites near water bodies⁶. Saprophytic fungi are quickly germinated and vital for the maintenance of natural ecosystem by breaking down of decomposes into small organic molecules, which can be absorbed by decomposers or nearby plants and other microbial flora⁷. It was reported that *Aspergillus* spp. is most dominantly contributing and the other dominant spores are *Fusarium* spp., *Curvularia* spp. and *Cladosporium* spp. in environment⁸.

Present piece of work was focused on isolation of fungal flora grown on polythene debris around water bodies in Jhansi due to potential of fungal strains and their degradability, because fungi are versatile organisms, able to grow and degrade a variety of compounds, organic contaminants and polymeric materials⁹. Enzyme activity of biodegradation is higher in fungi than in bacteria. Isolation of different fungal strains were made from submerged polythene with heavy fungal growth at decaying sites in Jhansi. A variety of species were found. Fungal isolation and identification from polythene is never testified before in Jhansi.

Research location

Degraded polythene sample were collected from different water bodies of Jhansi as given below (Table 1). Which is located in 25°26'55,03"N, 78°34'10,63"E, Lat: 25.4441, Long: 78.5676. It has an average elevation of 284 meters (935 feet). Jhansi lies on the plateau of central India, an area dominated by rocky relief and minerals underneath the soil. Average annual minimum and maximum temperature range is from 4°C to 46°C.

Table 1. List of different water bodies

S. No.	Habitats	No. of sample	No. of fungal isolates
1	Parichha dam	2	3
2	Antia pond	4	6
3	Pahuch dam	2	4
4	Betwa river	4	5
5	Orachha river	3	2
6	Sukamadukama dam	4	3
7	Pahuch canal	4	2

Methodology

Samples were collected from different water bodies (dumping sites) of Jhansi city. These samples were further treated for fungal

isolation. Samples were inoculated and plated on PDA plates and sub cultured for pure fungal isolates. Fungi were identified by microscopic examination¹⁰.

Results and Discussion

Sub-cultured isolates were identified using microscopic observation and using Lactophenol cotton blue stain. Ten isolates were identified; on the basis of their morphological and cultural characteristics, observed and noted after 7 days of incubation. These fungi were identified as *Penicillium chrysogenum*, *Rhizopus nigricans*, *Chaetomium murorum*, *Memmoniella echinata*, *Aspergillus fumigatus*, *Stachybotrys chartarum*, *Aspergillus niger*, *Chaetomium globosum*, *Aspergillus flavus* and *Fusarium oxysporum*

1. *Penicillium chrysogenum*

Division: Ascomycota
 Class: Eurotiomycetes
 Order: Eurotiales
 Family: Trichocomaceae
 Genus: *Penicillium*
 Species: *chrysogenum*

Colony is cottony, rapid growth, conidium green and diameter of colony was around 35mm. Conidia 2.8x2.5 μm , Subglobose, Smooth, Finely roughened (Plate-1, Fig. A) Stripe was 258x3.4 μm . Phialide was flask shaped and branching pattern was quarteverticillate¹¹.

2. *Rhizopus nigricans*

Division: Zygomycota
 Class: Mucormycotina
 Order: Mucorales
 Family: Mucoraceae
 Genus: *Rhizopus*
 Species: *nigricans*

Colony is white, cottony, rapid growth. Hyphae broad, scarcely septate, rhizoids and stolons present. Sporangioophores were brown. Rhizoid length was 300-350. Sporangioophore length was 1500-4000. Sporangium length was 150-350 (Plate-1, Fig. B). Columellae were almost round. Sporangiospores were variable in size,

average length 9-11 μm ; elongated to polyhedric¹².

3. *Chaetomium murorum*

Division: Ascomycota
 Class: Sordariomycetes
 Order: Sordariales
 Family: Chaetomiaceae
 Genus: *Chaetomium*
 Species: *murorum*

Colony was bluish gray to black in color, with the surrounding agar was yellowish brown. Perithecia dark colored, globose to subglobose, 260-320 X 250-300 μ ostiolate. Terminal hair dark olive brown, slender, gracefully flexed, with open circinate, blunt tips, 5.5-6 μ wide, smooth septate (Plate-1, Fig. C). Lateral hairs olive brown below, fading above, straight to flexed, with pointed tips, smooth, septate, 4.5-5 μ wide at the base. Asci was club-shaped, 8-spored. Ascospores dark olive green, ellipsoid, 12-13.5 X 6-7.5 μ , subapiculate at 1 or both ends¹³. The genus *Chaetomium* was established by Kunze and Schmidt¹⁴ but further redefined on several occasions and discovers many other species^{13, 15-17}.

4. *Memmoniella echinata*

Division: Ascomycota
 Class: Sordariomycetes
 Order: Hypocreales
 Family: Hypocreales
 Genus: *Memmoniella*
 Species: *echinata*

Colonies small, thick, white-black composed of hyaline septate mycelium. Conidiophores were blackish, erect, hyaline at the base 55-85 μm , long, 3-4 μm wide. Conidiophores bear one or two compact whorls of about ten phialides (Plate-1, Fig. D). Phialides sub hyaline, one celled, about 7-9 μm long, 2 μm wide, slightly diverging. Conidia opaque black, globose rough or somewhat angular 6 μm to 8 μm wide and 4 μm thick, forming

persisting long chain of conidia up to 150 μm long dark in color¹⁸.

5. *Aspergillus fumigatus*

Phylum: Ascomycota
 Class: Eurotiomycetes
 Order: Eurotiales
 Family: Trichocomaceae
 Genus: *Aspergillus*
 Species: *fumigatus*

Colonies cottony, velvety, green-brown with a white apron at the margin. The reverse color is usually white to tan unlike most aspergillus species. *A. fumigatus* grown at 45° and conidiophores are long 300-500 μm , have club-shaped vesicles that are 30-50 μm in diameter (Plate-1, Fig. E). Vesicles are uniseriate and are covered by conidia on only the distal half conidia arise in chain and tend to sweep toward the central axis¹⁹. Konduri *et al.*²⁰ was also worked on many *Aspergillus spp.* and reported that *Aspergillus spp.* can grow to 72% reduction in percentage of elongation in polythene.

6. *Stachybotrys chartarum*

Division: Ascomycota
 Class: Sordariomycetes
 Order: Hypocreales
 Family: Stachybotryaceae
 Genus: *Stachybotrys*
 Species: *chartarum*

Stachybotrys chartarum is also known as *Stachybotrys atra* and *stachybotrys alternans*. Colonies are thick dark black. Mycelium hyaline, almost hyaline towards base, septate, fuliginous near tip, 73-91 μm long, 13-16 μm wide. Conidiophore terminating in 3-5 cluster of phialides. Phialides 70-90 μm long X 4-6 μm in diameter (Plate-1, Fig. F). Conidia single, aseptate, smooth, ellipsoidal to broadly ellipsoidal, dark colored 8-16 X 3-9 μm diameter¹³.

7. *Aspergillus niger*

Division: Ascomycota
 Subphylum: Pezizomycotina

Class: Eurotiomycetes
 Order: Eurotiales
 Family: Trichocomaceae
 Genus: *Aspergillus*
 Species: *niger*

Colonies start white to pale yellow but quickly formed jet-black conidia, the reverse color is usually buff or yellow – gray. Characteristically obscure metulae and phialides, conidia are spherical, 3-5 μm , and roughen with maturity (Plate-1, Fig. G)²¹.

8. *Chaetomium globosum*

Division: Ascomycota
 Class: Sordariomycetes
 Order: Sordariales
 Family: Chaetomiaceae
 Genus: *Chaetomium*
 Species: *globosum*

Colony size is 7-8 mm, with pale or olivaceous aerial mycelia. Ascospores mature within 7-9 days, measured 175-280 μm , and were olivaceous, gray- green or brown in reflected light and tended to be superficial, spherical, ovate or obovate and ostiolate. The ascospore wall was brown in color and composed of texture intricate. The cells were 2.0-3.5 μm in breadth and ascospore hairs were numerous, typically unbranched, flexuous, undulate or coiled, often tapering, septate, brownish, 3-4.5 μm in breadth at base and up to 500 μm in length. The asci were clavate or slightly fusiform, stalked, evanescent, measured, 30-40 X 11-16 μm , and contained eight ascospores (Plate-1, Fig. H). Ascospores were limoniform, typically biapiculate, bilaterally flattened, brownish when mature, thick walled, contained numerous droplets, measured 9-12 X 8-10 X 6-8 μm , and featured an apical germ pore¹⁴.

9. *Aspergillus flavus*

Division: Ascomycota
 Class: Eurotiomycetes
 Order: Eurotiales
 Family: Trichocomaceae

Genus: *Aspergillus*

Species: *flavus*

Conidiophores were heavy walled, uncoloured, coarsely roughened, usually less than 1mm in length. Vesicles are elongate when young, later becoming subglobose or globose, varying from 10-65 μ m in diameter (Plate-1, Fig. I). Phialides were uniseriate or biseriate. The primary branches were up to 10 μ m in length and secondary 5 μ m. Conidia were typically globose to subglobose, varying from 3.5 to 4.5 μ m diameter²².

10. *Fusarium oxysporum*

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: *Fusarium*

Species: *oxysporum*

The coloration of *Fusarium oxysporum* mycelium was white. Their conidiophores, means through which *Fusarium oxysporum* asexually reproduce, were short, single, lateral monophialides (flask-shaped projections) in the aerial mycelium, later arranged to densely branched clusters. Their macro-conidia were fusiform, slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 x 3-4.5 μ m (Plate-1, Fig. J). Micro-conidia were abundant, never in chains, mostly non-septate, ellipsoidal or cylindrical, straight or curved, 5-12 x 2.3-3.5 μ m. chlamydospores were terminal or intercalary, hyaline, smooth, 5-13 μ m²³.

Materials those are most susceptible to mold attacks such as wood jute etc., were water damaged, in which molds most frequently encountered were *Penicillium* (68%), *Aspergillus* (56%) and *Chaetomium* (22%)²⁴. Water ecosystem is very rich in microbial diversity, only less than 5% of species have been described²⁵.

Fungi are widely found organism, grown rapidly. Fungi have the strong proficiency to degrade lignin, cellulose, starch and other organic material and consumed it as nutrition²⁶. This blissful nutrient strategy is base of biodegradation. Natural decaying site in Jhansi are good source of microbial flora, environmental condition is favorable for rapid fungal growth. Samples were collected with heavy fungal growth. 10 strains were isolated, out of which 9 were from Ascomycota and one was of Zygomycota. Similar to this study, Gilan *et al.*²⁷ was also worked on different fungal species with starch blend polythene and found that degradation rate is directly proportional to starch content present in polythene. Konduri *et al.*²⁰ was also investigated on many *Aspergillus spp.* and reported that *Aspergillus spp.* can grow to 72% reduction in percentage of elongation in polythene. *Chaetomium spp.*, *Rhizopus spp.*, *Penicillium spp.*, *Aspergillus spp.* are saprophytic fungi and economically beneficial. The genus *Chaetomium* was established by Kunze and Schmidt²⁸ but further redefined on several occasions and discover many other species^{13,15-17}. *C. Globosum* is recognized as a cellulolytic and endophytic fungus, known for having great potential in agricultural, medicinal and industrial fields²⁹. Some collected samples were even found fragile, brittle and in crumble situation with very heavy fungal growth. Fungi can grow everywhere except in under water. The atmosphere of Jhansi is appropriate for fungi. Previously, Raaman *et al.*⁶ conduct the similar study near Chennai and reported *Aspergillus niger*, *A. japonicus*, *A. terreus*, *A. flavus* and *Mucor sp.* and reported 8% degradation in one month period by *A. niger*. Some excellent studies was also carried out the tremendous variety of fungal flora grown on polythene on

lignocellulose, PHB, PCL, PBS, PBS/A, Poly[3HB-co-(12 mol%) 3HV] in last two decades- *Chaetomium globosum*, *Rhizopus* spp., *Fusarium oxysporum*, *Mucor* spp.,

Cryptococcus Laurentii, *Penicillium* Spp., *Curvularia* Spp., *Aspergillus fischeri*, *Gliocladium virens* etc^{9,29-34}. Environment of Jhansi is highly suitable due to

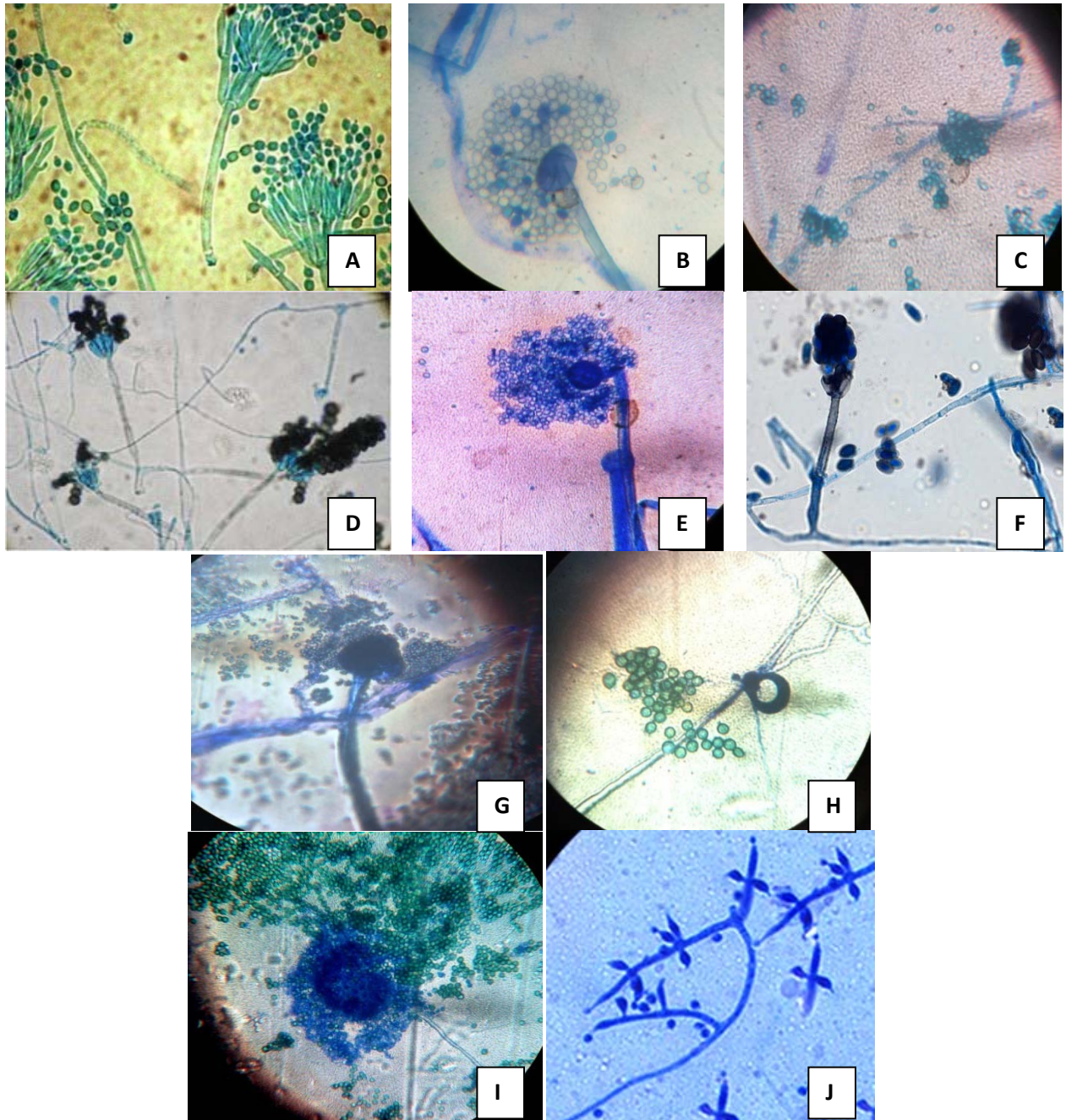


PLATE-1 (A) *Penicillium chrysogenum* (B) *Rhizopus nigricans* (C) *Chaetomium murorum* (D) *Memmoniellae chinata* (E) *Aspergillus fumigatus* (F) *Stachybotrys chartarum* (G) *Aspergillus niger* (H) *Chaetomium globosum* (spores) (I) *Aspergillus flavus* (J) *Fusarium oxysporum*

temperature range from 10-45° C where many fungal species may be found abundantly but this is first time these fungi were testified from Jhansi region.

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