J. Phytol. Res. **30** (1) : 7-15, 2017 **ISSN 0970-5767**

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

ANKITA SAXENA and ARVIND PAREEK*
Department of Microbiology, Mewar University, Gangrar, Chittorgarh, 312901, India
* Department of Botany, Maharshi Dayanand Saraswati University, Ajmer, 305009, India
* Corresponding author : E-mail: arvindmdsu@gmail.com

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 gas[1]. Polythene are nonmetallic moldable compounds that can be pushed into almost any desirable shape and then retain in that shape [2]. 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 dioxins[3]. 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 drains[4]. 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 pollutant[5].

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 bodies[6]. 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 flora[7]. It was reported that *Aspergillus* spp. is most dominantly contributing and the other dominant spores are *Fusarium spp*., *Curvularia spp. and Cladosporium spp.* in environment[8].

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 materials[9]. 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 examination[10].

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, Memnoniella 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 quarteverticillate[11].

2. Rhizopus nigricans

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

Colony is white, cottony, rapid growth. Hyphae broad, scarcely septate, rhizoids and stolons present. Sporangiophores were brown. Rhizoid length was 300-350. Sporangiophore length was 1500-4000. Sporangium length was 150-350 (Plate-1, Fig. B). Columellae were almost round. Sporangiospores were variable in size, average length 9-11µm; elongated to polyhedric[12].

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 surronding agar was yelloish 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 6-7.5µ, subapiculate at 1 or both ends [13].The genus *Chaetomium* was established by Kunze and Schmidt[14] but further redefined on several occasions and discovers many other species[13, 15-17].

4. Memnoniella echinata

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

Colonies small, thick, white-black composed of hyaline septate mycelium. Conidiophores were blackish, erect, hyaline at the base 55-85µm, long, 3-4µm 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, 2µm 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 color [18].

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 µ, have club-shaped vesicles that are 30-50 µ 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 axis[19]. 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: Sordariomycates
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 µ long, 13-16 µ wide. Conidiophore terminating in 3-5 cluster of phialides. Phialides 70-90 µ long X 4-6 µ in diameter (Plate-1, Fig. F). Conidia single, aseptate, smooth, ellipsoidal to browdly ellipsoidal, dark colored 8-16 X 3-9 µ diameter[13].

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 obscurus metulae and phialides, conidia are spherical, 3-5 µ, and roughen with maturity (Plate-1, Fig. G)[21].

8. ***Chaetomium globosum***

Division: Ascomycota
Class: Sordariomycates
Order: Sordariales
Family: Chaetomiaceae
Genus: *Chaetomium*
Speices: *globosum*

Colony size is 7-8 mm, with pale or olivaceous aerial mycelia. Ascomata mature within 7-9 days, measured 175-280µm, and were olivaceous, gray- green or brown in reflected light and tended to be superficial, spherical, ovate or obovate and ostiolate.The ascomatal wall was brown in color and composed of textura intricate. The cells were 2.0-3.5 µm in breadth and ascomatal 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 pore[14].

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 mm in diameter (Plate-1, Fig. I). Phialides were uniseriate or biseriate. The primary branches were up to 10 mm in length and secondary 5mm. Conidia were typically globose to subglobose, varying from 3.5 to 4.5 mm diameter[22].

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 µm (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 µm. chlamydospores were terminal or intercalary, hyaline, smooth, 5-13 µm[23].

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 described[25]. Fungi are wildly found organism, grown rapidly. Fungi have the strong proficiency to degrade lignin, cellulose, starch and other organic material and consumed it as nutrition[26]. 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 wasof 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 Schmidt[28] but further redefined on several occasions and discover many other species[13,15-17]. *C. Globosum* is recognized as a celluloltic and endophytic fungus, known for having great potential in agricultural, medicinal and industrial fields[29]. 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 decates- *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

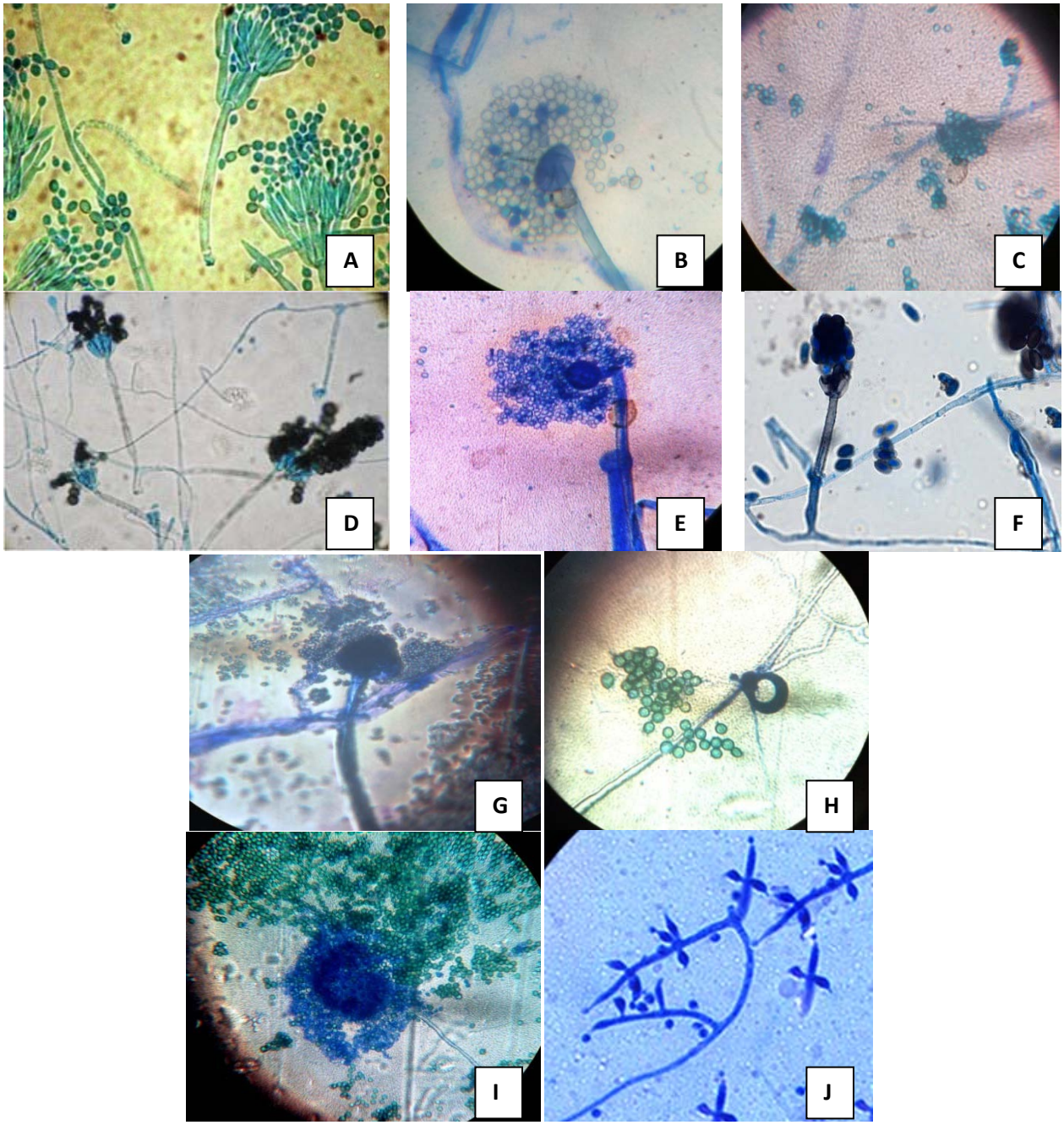

PLATE-1 (A) *Penicillium chrysogenum* (B) *Rhizopus nigricans* (C) *Chaetomium murorum* (D) *Memnoniellae chinata* (E) *Aspergillus fumigatus* (F) *Stachybotrys chartarum* (G) *Aspergillus niger* (H) *Chaetomium globosum (spores)* (I) *Aspergillus flavus* (J) *Fusarium oxysporum*

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

Reference

1. Mukherjee S and Chatterjee S 2014, A comparative study of commercially available plastic carry bag biodegradation by microorganisms isolated from hydrocarbon effluent enriched soil. *Intern. J Curr.Micro. Appl. Sci*. **3**(5) 318-325.
2. Seymour R B 1989, Polymer Science Before and After 1899: notable developments during the life time of Mautis Dekker. *J. Macromol Sci. chem*. **26** 1023- 1032.
3. Ojha N, Pradhan N, Singh S, Barla A, Shrivastava A, Khatua P, Rai V and Bose S 2017, Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. *Scientific reports*.**7**, 39515. DOI: 10.1038/srep39515.
4. Roff W J and Scott J R 1971, Fibers, film, plastics and rubbers, Butterworth's, London, 66-71.
5. Sangale M K, Shahnawaz M and Ade A B 2012, A review on biodegradation of polythene: *The micro.appro. Biorem.Biode*. **3**(10) 164. DOI: 10.4172/2155-6199.1000164.
6. Raaman N, Rajitha N, Jayshree A and Jegadeesh R 2012, Biodegradation of plastic by *Aspergillus Spp.* Isolated from polythene polluted sides around Chennai. *J. Acad. Indus. Res*. **1**(6) 313-316.
7. Bandh S A, Kamili AN and Ganai B 2012, Identification of some *penicillium* species isolated from Dal Lake, Kashmir by traditional approach of morphological observation and culture. *Afri. J Micro. Res*. **6**(29) 5824-5827.
8. Verma K S and Bhandari S 1992, Incidence of *Aspergillus* inside poultry sheds at Jabalpur. *Ind. J.Aerobiol*.**5** 46-48.
9. Chiellini E,Corti A, D'antone S and Solaro R 2003, Biodegradation of poly (vinyl alcohol) based materials. *Prog.Polymer.Sci*. **28** 963-1014.
10. Harris J L 1986, Modified method for fungal slide culture. J. *Clin. Microbiol*. **24** 460-461.
11. Thom C 1910, Cultural studies of species of *Penicillium* in USDA. *Bureau of animal industry bulletin*. **118** 1-109.
12. Gilman J C 1957, A manual of soil fungi. The Iowa State University Press. Iowa USA.
13. Corda A C J 1837, Icones fungorum hucusque cognitorum.(Eds) J. G. Calve (Prague). **1**,1-32.
14. Kunze G 1830, Uredo (Ustilago) trichophora β penniseti kunze; n.spp. In Holl Fungi. verzeichnis der auf der insel Madeira beobachteten pflanzen, nebst Beschrei bungeini gerneuen Arten. Flora **13**, 369-392.
15. Fries E M 1849, Summa vegetabilium scandinaviae, 2. Stockholm and Leipzig. Bonnier.
16. Chivers A H 1912, Contributions from the cryptogamic laboratories of Harvard University. No. LXVIII. Preliminary diagnoses of new species of *Chaetomium. Proceedings of the American Academy of Arts and Sciences.* **48**(4), 83-88. DOI: 10.2307/20022812.
17. Seth H K 1970, A monograph of the genus *Chaetomium*. *Beiheftezur Nova Hedwigia*. **37**, 1-133.
18. Galloway 1933, Memnoniella echinata. *Trans. Br. Mycol. Soc*. **18**,

163-166.

19. Schmidt A and Schmidt D I 1999, JB Georg W Fresenium and the description of the species *Aspergillus fumigatus*in 1863. *Contrib Microbiol*. **2**, 1-4.
20. Konduri M K R, Anupam K S, Vivek J S, Kumar R D B and Narasu M L 2010, Synergistic Effect of Chemical and Photo Treatment on the Rate of Biodegradation of High Density Polyethylene by Indigenous Fungal Isolates. *Inte.jbiote. bioch*. **6** 157-174.
21. Gomez R, Schnabel I and Garrido J 1988, Pellet growth and citric acid yield of *Aspergillus niger*. *Enzyme Microb Technol*. **10** 188-191.
22. Link H F 1809, Observations in Ordines plantarum naturales. Dissertatio prima, complectens Anandrarumordines Epiphytas, Mucedines Gastomycoset Fungos Der Gesellschaft Naturforschender Freundezu Berlin. *Magazinfu¨r die neuesten Entdeckungen in der gesamten Naturkunde*. **3** 1–42.
23. Fisher N L, Burgess LW Toussoun, T A and Nelson P E 1982, Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*. **72** 151–153.
24. Gravesen S, Nielsen P A, Iversen R and Nielsen K F 1999, Microfungal contamination of damp buildings--examples of risk constructions and risk materials. *Environ Health Perspect*. **3** 505-508.
25. Thatoi H, Behera B C, Mishra R R and Dutta S K 2013, Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: A review. *Ann microbial*. **63** 1-19. DOI: 10.1007/s13213-012-0442-7.
26. Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De la Fuente G and Falcon M A 1996, Degradation of natural lignins and lignocellulosic substrate by soil-inhabiting fungi imperfective. *FEMS microbial. Ecol*. **21** 213-219.
27. Gilan I, Hadar Y and Sivan A 2004, Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcusruber*. *Appl. Microbiol.Biotech*. **65**, 97-104.
28. Kunze G and Schmidt J K 1817, Chaetomium globosum kunze, *Mykol Hefte*. **1**(16).
29. Wang X W, Lombard L, Groenewald J L, Videira R A, Samson X Z L and Crous P W 2016, Phylogenetic reassessment of the *Chaetomium globosum* species complex. *Persoonia*. **36**, 83-133. DOI: Org/10.3767/003158516x689657.
30. Zhang J, Wang X, Gong J and Gu Z A, 2004, Study on the biodegradability of polyethylene terephthalate fiber and diethylene glycol terephthalate. *J appl. Poly.Sci*. **93** 1089–1096.
31. Suhail M, Irum F, Jatt T, Korejo F and Abro H 2007, *Aspergillus mycoflora* isolated from soil of kotri barrage Sindh, Pakistan. *Pak. Jour. Botany*. **39**(3) 981-984.
32. Sanchez C 2009, Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotech. Adv*. **27** 185-194. DOI:10.1016/j.biotechadv.2008.11.001
33. Sang B I, Hori K, Tanji Y and Unno H 2002, Fungal contribution to in situ biodegradation of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) film in soil. *Appl Micro Biotechnol*. **58** 241–247.DOI: 10.1007/s00253-001-0884-5.

34. Jain K 2000, Isolation of microorganisms causing biodegradation of plastics, Ph.D thesis. Thaper University, Panjab.