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Microbial diversity in grassland shola forests of Wayanad and Munnar

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CONTENTS

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ABSTRACT

Soil microbial diversity and fungi involved in litter decomposition was studied for a period of two years in the shola forests of Munnar and Wayanad. The sholas selected were Mannavan shola, Pambadam Shola and Manthan shola in Munnar Forest Division and Meppadi shola and Brahmagiri shola in Wayanad Forest Division. The density of fungal population varied between sholas. The population of fungi was highest in Pambadam shola and lowest in Manthan shola. Both in Pambadam and Manthan shola the bacterial population was higher at a soil depth of 0-10 cm. The actinomycete population was also high in Manthan shola at 0-10 cm and 10-20 cm depth. In Manthan shola, the fungal density of fully decomposed leaf was low. *Aspergillus, Penicillium, Trichoderma, Verticillium* and *Pestalotiopsis* were the common dominant genus identified from all sholas. Among the actinomycetes, the genus *Streptomyces* dominated in all sholas. Seventeen species of fungi are new records to Kerala.

1. INTRODUCTION

The tropical forests are the richest biome which supports about 1,00,000 higher plants (*i.e*) about 40% of all described higher plants of the world (Nayar, 1997). About two-third of the total populations of plants are believed to occur in the tropics (Raven, 1988). The tropical forest is considered to house the greatest biodiversity on earth. It is estimated that there are 5-30 million species of organisms on earth; less than 1.4% of which have been described (May, 1988). Ehrlich and Ehrlich (1992) reported that a gram of forest soil in the tropics contain more than a million bacteria, about 1,00,000 yeast cells and about 50,000 fungi.

A comparison of the number of known and estimated total species of microorganisms in the world indicated that 15% of fungi, 78% of bacteria and 96% of viruses still remain to be discovered (Bull *et al*. 1992). An analysis of the newly described fungi during 1981-90 revealed that around 50% were discovered in the tropics (Hawksworth, 1993). Though the magnitude of biodiversity is great, the tropics are totally unexplored for microorganisms compared to other parts of the world (May, 1988; Hawksworth, 1991). The information on the density and diversity of fungi, bacteria and actinomycetes occupying the various ecological regions in the tropical forests are very scarce. The present study is an attempt to estimate the microbial population and identify fungi and actinomycetes occurring in shola forests of Wayanad and Munnar.

Shola forests as redefined by Meher - Homji (1968) includes forest vegetation of the Peninsular India growing above 1,500 m asl approximately. These forests are unique vegetation occupying temperate habitats in tropical latitudes and are regarded as relict communities. They are high altitude gallery forests restricted to valleys, depressions and especially along folds of hills and watercourses. Shola forests are considered to be ecologically unique since they harbour many endemic species. The extent of shola forests in Kerala accounts to 70 km^2 (Raghavan Nair, 1997). They are reported to contain very high floristic richness and diversity, probably the highest in the Western Ghat region (Jose *et al*. 1994). The characteristically deep fertile soils and high moisture holding

capacity of these forests offer excellent conditions for the proliferation of a large number of microorganisms. Sholas are interesting as a special ecological type due to the prevalence of unique micro and macro climatic conditions. Only scanty information is available on the floral diversity, vegetation structure and dynamics of shola forests. The knowledge of the soil microbial diversity of shola forests is very little and the microbial flora of shola forests remains virtually unexplored. So far no attempts have been made to understand the diversity of microbes in this ecosystem except the study conducted by Sankaran *et al*. (2000) in Eravikulam National Park. The present study was intended to investigate the microbial diversity in shola forests of Wayanad and Munnar. The specific objectives of the project are:

To prepare an inventory of the soil microorganisms (Fungi, bacteria and actinomycetes) in the shola forests of Wayanad and Munnar

To study the fungal flora associated with leaf litter in the shola forests.

To prepare and maintain pure cultures of fungi for future biotechnological uses.

2. MATERIALS AND METHODS

Three sholas were selected for the study in the Munnar region namely Mannavan shola, Manthan shola and Pambadam shola. In Wayanad region, Meppady and Brahmagiri shola were selected for the study. From each shola three sites were selected randomly for microbial enumeration study.

Mannavan shola

Mannavan shola forms the largest shola forest patch in Kerala State with an approximate area of 5.18 km^2 (Swarupanandan, 1998). Mannavan shola comes under the Marayoor Forest Range of Munnar Forest Division is located between $10^0$10' 00 and 10^0 12' 18" North latitudes and 77^0 9' 50" and 77^0 12' 18" East longitudes. The altitudes ranges between 1600 to 2400 m asl. The other major sholas, Pambadam shola, Manthan shola, Pullardi shola and Idivara shola lie adjacent to Mannavan shola. The vegetation comprises mostly of Southern Sub-tropical Forests which gradually transform to the Southern Montane Temperate Forests (Champion and Seth, 1968). Mean annual temperature is about 20 ^{0}C and the coldest months are December and January when the minimum temperature goes down up to 5-6 ^{0}C. The annual rainfall ranges between 2000 – 3000 mm.

Meppady shola

Meppady is located between 76 ^{o} 4' and 76 ^{o} 7' East latitudes and 11^o 31' and 11^0 35' North longitudes. The altitude ranges between 1100 to 1800 m asl. The present study was carried out in Chembra hills. The forest type of Chembra hills range from tropical wet evergreen to montane subtropical forest, associated with vast areas of grasslands. The shola patches were seen from about 1500 m asl extending up to 1800 m asl. The vegetation was characterised by trees, shrubs, ferns and orchids.

Brahmagiri shola

Brahmagiri shola is situated in Begur Range of Wayanad North. Begur Range lies between 75^{o} 54' to 76^{o} 5' E and 11^{o} 40' to 11^{o} 57' North. Shola occupies in the northern part of the Thirunelly temple. The altitude of the shola ranges from 700 to 1600 m asl.

Three study sites were selected randomly for the collection of samples from each shola. During each sampling, soil samples were collected at a depth of 0-10 cm and 10-20 cm. For collecting 0-10 cm depth, the top layer of the litter was removed and soil cores were taken using soil tubes. Four samples at two soil depth were collected from each plot in each shola during March - April for two consecutive years (1999-2000). Immediately after bringing the soil samples to the laboratory, pH of the soil sample was measured using pH meter and soil moisture determined by oven dry method. The rest of the soil samples were stored at 4^{0}C, till they were processed for isolation of fungi, bacteria and actinomycetes, Soil dilution plate method (Waksman, 1922; Timonin, 1940) was used for isolation of microorganisms. For isolations, 10 g of a working sample from each composite sample was transferred to 100 ml sterile water in 250 ml conical flasks. The samples were shaken thoroughly and appropriate dilutions were prepared ie. 10^{3} for fungi, 10^{4} for actinomycetes and 10^{5} for bacteria. Potato dextrose agar (PDA) and Rose Bengal agar (RBA) media were used to isolate fungi (Fig. 1), while starch casein agar (SCA) and soil extract agar (SEA) were used to isolate actinomycetes and bacteria respectively. One ml of the soil suspension was transferred to individual Petri plates and appropriate media added. Five replicate Petri dishes were used for each medium. The Petri plates were incubated in the dark and colonies of different microorganisms enumerated after appropriate time interval (48 h for bacteria, 6 days for fungi and 10-14 days for actinomycetes). These data were used to

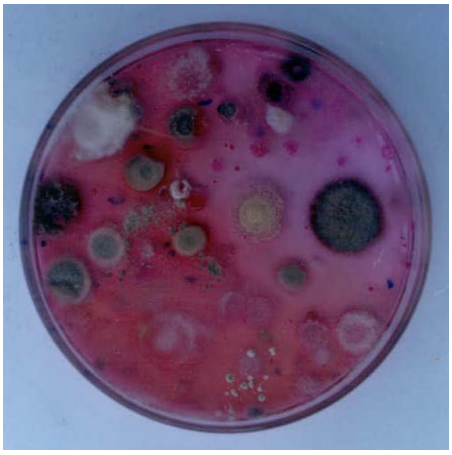

Fig.1. Fungi isolated using Rose Bengal agar

compute microbes per gram of oven dry soil. The fungal, bacterial and actinomycetes isolations were maintained on PDA, Nutrient agar (NA) and SCA slants respectively. As far as possible the fungi were identified up to species level.

Three different types of leaf litter such as freshly fallen, partially decomposed and fully decomposed were collected from each sample plot for isolation of fungi associated with leaf litter. The leaf litter was powdered using a sterile blender and this powder was used for the isolation of fungi. For isolations, 10 g of the powdered sample was transferred to 90 ml sterile water in 250 ml conical flasks. The samples were shaken thoroughly and 10^4 dilution was prepared for isolation of fungi using Rose Bengal agar. Fresh leaves of dominant tree species were collected to study the phylloplane microorganisms associated with each shola. For the isolation of phylloplane fungi, the dorsal and ventral surfaces of the leaf were pressed separately on PDA using a sterile forceps for trapping the fungal spores on either side of the leaf. For each side of the leaf, triplicate Petri plates were maintained and incubated at 28 ± 2 ^{0}C. Leaf washings were also used to isolate the phylloplane fungi. Representative samples of leaves were washed in 100 ml of sterile water and 10^4 dilution was prepared and enumerated for phylloplane fungi using RBA. All the fungal colonies were subcultured and maintained on PDA for identification. Microbial isolations were also done from available seeds collected during the sampling. The standard blotter method for seed testing was employed (ISTA, 1966). The seeds were placed at equal distance in sterilized Petri dishes containing three moist filter paper disks. The number of seeds incubated per plate depended on the size of seeds. The Petri dishes were incubated at 28 ± 2 ^{0}C. Observations on the presence of microorganisms were recorded and the fungi colonised on the seeds were isolated and maintained in pure cultures.

The fungal cultures were identified referring the following books/monographs. Compendium of soil fungi by K H Domsch W Gams and Traute Heidi Anderson (1980), Vol. 1; The Genus *Penicillium* and its Telomorphic States *Eupenicillium* and *Thalaromyces* (1979), by John I Pitt; A Manual of the Penicillia (1968), by Kenneth B Raper and Charles Thom; Dematacеous Hyphomycetes (1971), by M B Ellis; The

Coelomycetes (1980), Brain C Sutton; Coelomycetous anamorph with appendage bearing conidia (1993), T R Nagaraj; A Revision of the genus Rhizopus (1984), by M A A Schipper; A manuel of soil fungi (1957), Joseph C Gilman; A revision of the genus *Trichoderma* (1969), M A Rifai; On certain species of *Mucor* with a key to al accepted species (1978), M A A Schipper and International course on the identification of fungi of agricultural importance (1994), International Mycological Institute, London.

3. RESULTS

The moisture content and pH of the soils collected from various sholas are given in Table 1. In general, the soils of all sholas were acidic in nature. There was slight increase in the pH of Manthan and Meppadi sholas (6.07 and 6.44). The moisture content varied with the change in the depth of soil. The upper layer of the soil (0-10 cm) contained more moisture than the lower layer.

Table 1. Moisture content and pH of soils of sholas in Wayanad and Munnar

Shola	Soil pH		Moisture content	
	0-10cm	10-20cm	0-10cm	10-20cm
Munnar region				
Mannavan shola	5.52	5.56	26.38	24.85
Pambadam shola	5.64	5.69	26.38	24.85
Manthan shola	6.07	6.00	30.60	28.77
Wayanad region				
Meppadi shola	6.44	6.36	38.95	32.93
Brahmagiri shola	5.84	5.76	28.39	23.06

The density of fungal population varied between different sholas (Table 2). The fungal population at a depth of 0-10 cm in Pambadam shola was the highest (44.5×10^4). When compared to other sholas, the fungal population was less in Manthan shola. In all sholas, at soil depth of 10-20 cm, the fungal density was comparatively low when compared to the soil depth of 0-10 cm. This is because the major organisms involved in decomposition are present in the upper layers of soil. Both in Pambadam shola and Manthan shola the bacterial population was more at a soil depth of 10-20cm. At a soil depth of 1-10 cm, the population of bacteria was more in Manthan shola and Meppadi shola. The actinomycete population was also high on Manthan shola at both soil depths. When compared to all sholas, the bacterial and actinomycete population was more in Manthan shola where as the fungal population was low.

Table 2. Polulation of fungi, bacteria and actinomycetes (10^4/gm oven dry soil) in shola forests of Wayanad and Munnar

Study plots	Fungi		Bacteria		Actinomycetes	
	0-10cm	10-20cm	0-10cm	10-20cm	0-10cm	10-20cm
Mannavan shola	42.08	35.08	42.83	34.83	5.00	7.00
Pambadam shola	44.40	33.58	32.58	144.13	16.40	-
Manthan shola	16.08	24.33	105.42	120.83	14.67	18.67
Meppadi shola	27.67	17.63	99.45	62.5	10.33	6.75

The population of fungi on leaves at different stages of decomposition was given in Table 3. The maximum number of fungi was associated with fully decomposed leaves. In Manthan shola, the fungal density of fully decomposed leaf was low. This is because the fungal population in soil was also less, as it is evident from the soil isolations (Table 2). In dry and partially decomposed leaves, no significant difference in fungal population was noted among various sholas.

Table 3. Population of fungi (10^4/gm oven dry weight) at different stages of decomposition

Study plots	Dry leaves	partially decomposed	Fully decomposed
Mannavan shola	41.67	42.33	52.33
Pambadam shola	26.11	34.56	42.33
Manthan shola	49.78	38.11	19.88
Meppadi shola	46.44	49.17	49.33
Brahmagiri shola	37.77	32.78	52.11

Fungi identified from various sholas in Munnar and Wayanad region were given in Table 4. *Aspergillus, Penicillium, Trichoderma, Fusarium, Verticillium and Pestalotiopsis* were the common dominant genus identified from all sholas. *Penicillium,* which contributed to the major species of the total, was the most dominant genus in shola forests (Fig.2).

Fig. 2 Different species of *Penicillium*

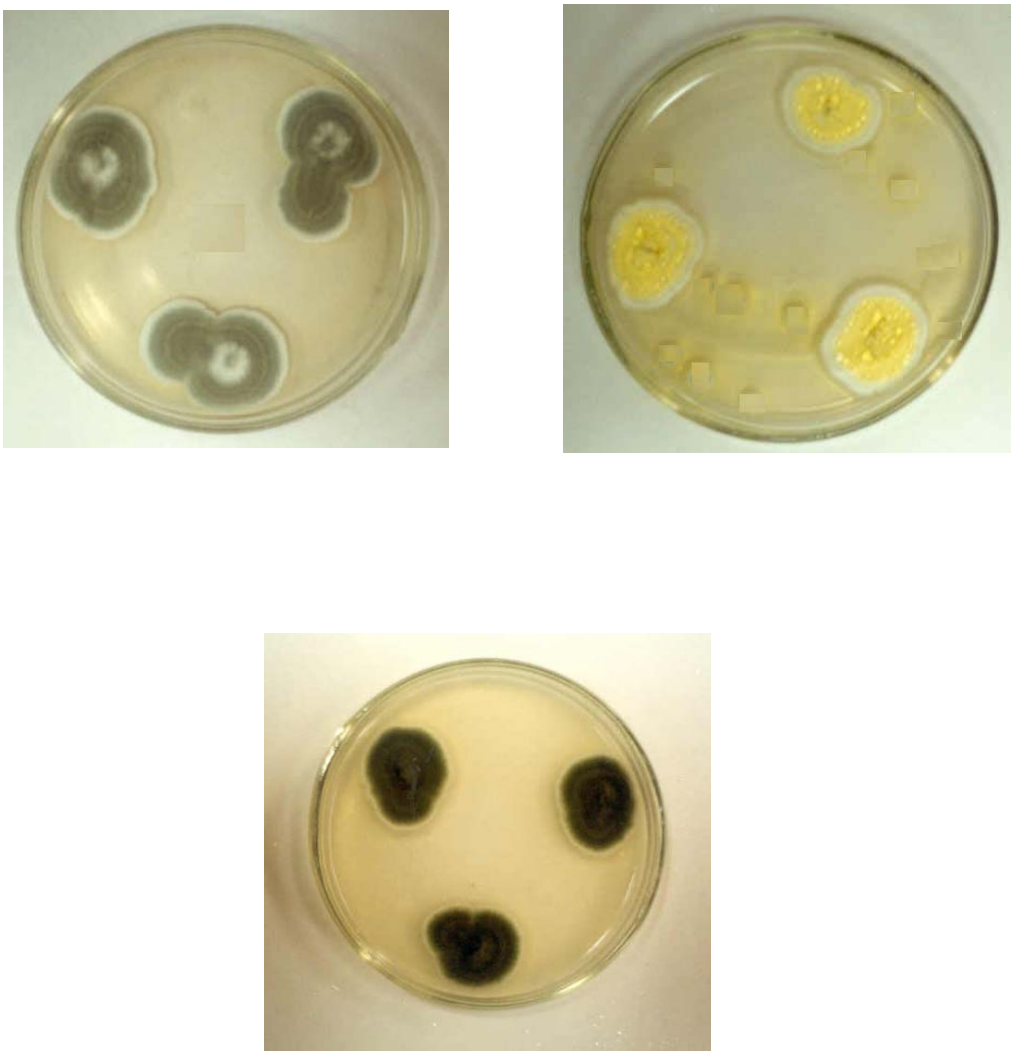

Table 4. Fungi identified from different shoals of Munnar and Wayanad

Mannavan shola	Meppadi shola	Pambadan shola	Manthan shola	Brahamigiri shola
Fusarium lateritium	*Cladosporium sphaerospermum*	*Fusarium stilbodies*	*Fusarium solani*	*Fusarium decemcellulare*
Mucor hiemalis f. sylvaticus	*Cylindrocladium camelliae*	*Fusarium sambusinum*	*Fusarium oxysporum*	*Aspergillus sp.*
Penicillium chermesium	*Cylindrocladium sp.*	*Curvularia lunata*	*Fusarium udum*	*Pencillium sp.*
Penicillium corylophylum	*Doratomyces stemonites*	*Gliocladium roseum*	*Fusarium equiseti*	*Pestalotiopsis sp.*
Trichoderma sp.	*Fusarium sp.*	*Trichoderma harzianum*	*Rhizopus stolonifer*	*Trichoderma sp.*
Cladosporium cladosporiodes	*Gliocladium roseum*	*Aspergillus sp.*	*Penicillium brevicompactum*	--
Aspergillus sp.	*Myrothecium roridum*	*Acremonium fusidiodes*	*Pencillium decumbans*	--
Colletotrichum acutatum	*Oidiodendron sp.*	*Cladosporium cladosporiodes*	*Pencillium crustosum*	--
Verticillim fungicola	*Pencillium sp.*	*Colletotrichum acutatum*	*Pencillium puberulum*	--
Penicillium sp.	*Pestalotiopsis sp.*	*Verticillium fungicola*	*Pencillium spinulosum*	--
Pestalotiopsis sp.	*Sesquicillium candelabrum*	*Pestalotiopsis uvicola*	*Trichoderma sp.*	--
--	*Staphylotrichum coccosporum*	*Pencillium sp.*	*Phoma glomerata*	--
--	*Trichoderma sp.*		*Pestalotiopsis sp.*	--
--	*Verticillium catenulatum*		*Aspergillus sp.*	--
--	*Verticillium catenulatum*		--	--
--	*Verticillium tricorpus*		--	--
--	*Wardomyces ovalis*		--	--
--	*Circinella sp.*		--	--

Fungi identified from different tree species growing in various sholas in Munnar region were listed in Table 5.

Table 5. Phylloplane/spermoplane fungi isolated from sholas in Munnar

Fungi	Host species	Isolated part
Mannavan shola		
Cladosporium cladosporioides	*Rhododendron nilgiricum*	leaves
Colletotrichum acutatum	"	leaves
Fusarium lateritium	*Transtroemia japonica*	seeds
Mucor haemalis f .silvaticus	*Hydnocarpus alpina*	seeds
Penicillium chermesinum	*Mastixia arborea*	leaves
Penicillium corylophilum	*Phohinia notorians*	seeds
Pambadam shola		
Acremonium fusidioides	*Litsea wightiana*	Leaves
Cladosporium cladosporioides	*Symplocos cochinchinensis*	Leaves
Colletotrichum acutatum	*Beilschmiedia wightii*	Leaves
Colletotrichum gloeosporioides	*(unidentified)*	Leaves
Curvularia lunata	*(unidentified)*	Leaves
Fusarium sambucinum	*Beilschmiedia wightii*	Leaves
Fusarium stilboides	*Acronychia laurifolia* *Litsea wightiana*	Leaves Leaves
Gliocladium roseum	*Acronychia laurifolia* *Piper mullesua* *Litsea wightiana*	Leaves Leaves Leaves
Pestalotiopsis uvicola	*Cinnamomum wightii*	Leaves
Trichoderma harzianum	*Actinodaphine bourdillonii*	Leaves
Verticillium fugicola	*Litsea wightiana* *Rhododendron nilgiricum*	Leaves Leaves
Manthan shola		
Fusarium equiseti	*Bhesa indica*	Leaves
F. oxysporum	*Psychotria sp.*	"
F. solani	*Psychotria nilgiriensis*	"
F. udum	*Clerodendron* sp.	"
Penicillium brevicompactum	*Rhododendron nilgiricum*	"
P. crustosum	"	"
P.decumbens	"	"
P. puberulm	"	"
P. spinulosum	"	"
Phoma glomerata	*Ilex thwaitesii*	"
Rhizopus stolonifer	*Bhesa indica*	"

The total number of bacteria was grouped on the basis of colony character, colour and Gram stain (Table 6).

Table 6. Bacteria grouped based on morphological characters

Sl. No	Colony characters					
	Gram staining	Shape	Colour	Colony shape	Colony surface	Colony edge
1	+	Round	Yellowish white	Circular	Smooth glistering	Entire
2	+	,,	,,	,,	,,	,,
3	+	,,	,,	,,	,,	,,
4	-	Rod	,,	,,	,,	,,
5	-	,,	Pastel red	Irregular	,,	Undulate
6	-	Round	,,	Circular	,,	Entire
7	-	,,	White	,,	,,	,,
8	-	Rod	,,	,,	Rough	Undulate
9	-	Round	Yellowish white	,,	Smooth glistering	Entire
10	+	Rod	Pale yellow	,,	,,	,,
11	+	Round	Yellowish white	,,	Smooth	,,
12	+	,,	White	,,	Smooth glistering	,,
13	+	,,	Pale yellow	,,	Smooth	,,
14	+	,,	,,	,,	Wrinkled	Undulate
15	-	,,	,,	,,	Smooth glistering	,,
16	+	Rod	Yellowish white	Irregular	Wrinkled	Undulate
17	+	Round	Pastel yellow	Circular	Smooth	Entire
18	+	Rod	Yellowish white	,,	Smooth glistering	Entire
19	+	Round	,,	,,	,,	,,
20	+	,,	,,	,,	Wrinkled	Entire

Most of the bacteria were positive to Gram stain. Based on the shape, cocci (round in shape) were more in number than bacilli (Rod shaped). More detailed studies are needed to identify the bacterial cultures.

The actinomycetes grouped based on the morphological characters were given in Table 7. Microscopic examination showed that the dominant genus of actinomycete in the soils of Munnar and Wayanad sholas was *Streptomyces.*

Table 7. Actinomycetes grouped based on morphological characters

Sl. No	Colony characters			
	Spore chain morphology	Surface colour	Reverse colour	Pigments & melanoids
1,	Rectiflexibiles	Violet grey (17E2)*	Yellowish white	Not produced
2,	Spirales	Dark brown (8F6)	Dark brown (8F6)	Produced
3,	Rectiflexibiles	Greyish grey (2C2)	Light yellow	Not produced
4,	Retinaculiaperti	Brownish grey (11C2)	Yellowish brown (5E5)	Soluble pigment produced
5,	Rectiflexibiles	Purplish grey (13E2)	Yellowish brown (5E7)	Produced
6,	Rectiflexibiles	Reddish white (10A2)	Red (9B7)	Not produced

* Methuen Handbook of Colour (1989)

The fungi isolated from the soil, leaf litter, phylloplane and spermoplane were identified using relevant literature and detailed description and distribution of the fungi recorded from various sholas are given below:

1. Acremonium fusioides (Nicot) W. Gams

Colonies on PDA slow growing, brown, powdery; ascomata absent in culture; conidia catenulate, in dry chains, brown, fusiform, 4-5x 1.8-2.5µm in size.

***Distribution*:** This is a widespread, but not a very common soil fungus. Recorded from Canada, Netherlands, Italy, India, Japan and Africa (Gams, 1971).

2. Aspergilllus niger van Tieghem

Colonies on MEA moderately growing, reaching 5-6 cm in ten days typically black powdery; conidiophores arising from long, broad, thick walled, mostly roughened, 4.0-

5.0µm diam (Fig. 6 *Aspergillus* sp.).

Distribution: The occurrence if this fungus is documented from all parts of the world. It is common in warmer regions (Domsch *et al.* 1980)

3. *Botryodiplodia theobromae* Pat.

Colonies grey to black, fluffy with abundant aerial mycelium, reverse black; conidiophores hyaline simple; mature conidia subovoid, uniseptate, longitudinally striated, hyaline and granulose.

***Distribution*:** This is a common weak wound pathogen of worldwide distribution and a common blue stain fungi (Punithalingam, 1980).

4. *Circinella* sp. Colonies on PDA fast growing, white, changing to light yellow; reverse yellowish white; mycelium strongly branched; lateral branches become more and nore delicate; are curved and carry sporangia at their tips; sporangia many spored, spherical; columella large; sporangiospores spherical to subspherical and smooth (Gilman, 1957) Fig. 4).

5. *Cladosporium cladosporioides* (Fres.) de. Vries

Colonies on PDA olive green, velvety; conidial scars prominent; conidiophores macronematous and slender; conidia smaller, smooth walled, elongated, nonseptate, measuring 5.4 - 9.9 x 2.7 µm.

***Distribution*:** Occurrence in tropical and subtropical zones. This species has been isolated from forests, grasslands, gardens, salt marshes and saline beaches (Park, 1972).

6. *Cladosporium sphaerospermum* Penz.

Colonies on PDA olive brown, reverse black; surface velvety; conidiophores macronematous, pale to dark olivaceous brown; not nodose; conidial scars prominent; conidia small, smooth, spherical 3-4µm diameter.

***Distribution*:** It is a cosmopolitan species found in air, soil and secondary invader of many plants (Ellis, 1971).

7 *Curvularia lunata* (Wakker) Boedijn.

Colonies on PDA not markedly zonate, olive grey, reverse brownish grey, floccose; conidia curved with hilum scarcely or not at all protuberant, smooth walled, predominantly 3 septate, middle septum not median; some conidial cells always mid dark brown 18-28 x 11-15µm in size.

***Distribution*:** Common and widespread on many different substratum (Ellis, 1971).

8. *Colletotrichum acutatum* Simmonds

Colonies on PDA pinkish grey; aerial mycelium white to grey, reverse dark pink; conidia straight, fusiform, 12-18 x 3-4µm in size.

***Distribution*:** This fungus was reported earlier from Australia and on *Pinus* seedlings from New Zealand (Dingley and Gilmour, 1972).

9. *Cylindrocladium camelliae* Venkataramani & VenkataRam

Colonies on PDA floccose, white, changing to light yellow, reverse yellow to light brown; penicillate as well as subverticillate type of conidiophores; stipes unbranched having basal septum only; measuring 82-115µm; phialides dolliform to slightly navicular 8-12 x 1.5-3µm; vesicle elongated, lanceolate, 13-32 x 4-6µm; conidia hyaline one septate 8-12 x 1.5-2µm in size.

***Distribution*:** This was reported from the leaves of *Acacia dealbata* in Japan (Terashita, 1969) and also from India (Venkataramani, 1952).

10. *Cylindrocladium sp.* Colonies in PDA floccose, yellowish brown, reverse brown, penicillate conidiophore, hyaline terminating in a clavate to ellipsoidal vesicle, 9.9 – 31.5 x 3.6 – 5.4 µm; stipe long 54.9 - 97.2 µm; phialides doliiform 9-15.3 x 2.3 – 3.2 µm; conidia 1 septate, cylindrical 7.2 – 11.7 x 1.8 – 2.7 µm in size (Fig. 9).

11. *Doratomyces stemonitis* (Pers. ex. Steud.) Morton & G. Sm.

Colonies on PDA membraneous to floccose, dark brown to black, producing synnemata which produces phialides; conidia formed in a chain like fashion, smooth, nonseptate, subspherical to cylindrical with truncate base and pointed apex, 5.5-7x3-4 µm in size.

***Distribution*:** Commonest species of its genus and has worldwide distribution. It has been reported from Germany, Czechoslovakia, Poland, USSR, Australia and Spain (Ellis, 1971). In India it was reported from sugar cane soils (Prakash and Khan, 1971).

12. *Fusarium decemcellulare* Brick

Colonies on PDA pale red; reverse ruby; growth rate above 2.5 cm diam; distinct microconidia abundant, formed in chains from simple phialides, subglobose to clavate 5-8 x 3-4µm; macroconidia 48-80 x 5-6µm.

***Distribution*:** This is a tropical fungus and has been reported on many trees (Booth, 1971).

13. *Fusarium equiseti* (Corda) Sacc.,

Colonies on PDA yellowish white, floccose, reverse greyish red; growth rate greater than 2.5-cm diam; No distinct microconidia present, heterogenous, formed from simple phialides; macroconidia with pedicellate foot cell, apical cell elongated in to an acicular tip, 2-5 septate, 18-36 x 2.7-4.5µm; intercalary and terminal chlamydospores present.

***Distribution*:** This is a common fungus in warm temperate and subtropical areas of the world (Booth, 1971).

14. *Fusarium lateritium* Nees.

Colonies on PDA exceeding 2.5 cm diam, floccose, yellowish white, reverse bright yellow; macroconidia usually present, formed from simple phialides; macroconidia with uncinate or hooked apical cell, 21-39 x 3-5 µm. in size.

***Distribution*:** This fungus has a wide host range, reported from Zambia, USA, USSR, Italy, Kenya, West Indies and Canada (Booth, 1971).

15. *Fusarium oxysporum* Schlecht.

Colonies on PDA white, floccose; growth rate greater than 2.5 cm diam; microconidia abundant, not formed in chains, formed from simple short lateral phialides, fusiform, measuring 4.5-11.7 x 1.8-2.7µm; macroconidia 16.2-24.3 x 2.7-4.5µm in size.

***Distribution*:** This has a worldwide distribution, mostly as a soil saprophyte in a wide

range of soils and numerous host plants (Booth, 1971).

16. *Fusarium sambucinum* Fuckel, var. *coeruleum* Wollenw.

Colonies on PDA floccose, greyish rose, reverse greyish ruby; growth rate above 2.5 cm diam; microconidia only produced on grouped simple phialides; phialides doliform to cylindrical, 8-10 x 2.5-3µm; macroconidia apically beaked, 1-4 septate, 24-30 x 3-4.5µm in size.

***Distribution*:** A canker causing organism of woody trees, reported from Europe, USSR, Australia and Malawi (Booth, 1971).

17. *Fusarium solani* (Mart.) Sacc.

Growth rate above 2.5 cm; culture pigmentation pale to yellowish white; microconidia distinct, generally abundant; not in chains; formed from simple phialides, elliptical to ovate, formed from well-developed microconidiophores; macroconidia ellipsoidal to oval.

***Distribution*:** Found on numerous plants, common occurrence in tropical and temperate regions (Wollenweber, 1935).

18. *Fusarium stilboides* Wollenw.

Growth rate above 2.5 cm; culture carmine red, with white floccose mycelium changing to yellow; reverse carmine red; microconidia absent; macroconidia variable in size, 3-8 septate, straight, with apical cell curved or beaked, 43.2-73.8 x 4.5-6.3µm in size.

***Distribution*:** Reported from Kenya, Tanzania, Malawi, Madagascar, West Indies, India and S. Africa (Booth, 1971).

19. *Fusarium udum* Butler

Colonies on PDA yellowish white with violet colouration at center; growth rate above 2.5cm diam; microconidia abundant, formed from simple phialides, curved, elliptical, allantoid, measuring 7.2-10.8 x 2.7-3.6µm; macroconidia 15.3-25.2 x 2.7-4.5µm in size.

***Distribution*:** This species causes a vascular wilt of *Cajanus cajan*, and has been reported from Germany, Italy, India, Tanzania and Vietnam (Booth, 1971).

20. *Gliocladium roseum* Bain.

Colonies on PDA white, reverse yellowish white; conidiophores differentiated into Verticillium like primary and Penicillium like secondary, conidiophore stipe smooth walled, primary conidiophores with divergent phialides; conidia mostly asymmetrical, navicular, 4.5-6.3 x.7-3.6µm in size. (Fig. 7).

***Distribution*:** In India, it was reported earlier from the rhizosphere of Tea (Agnihothrudu, 1961) and from the grasslands at Varanasi (Dwivedi, 1966).

21. *Mucor haemalis f. silvaticus* (Hagem) Schipper

Colonies on PDA yellowish white; sporangiophores 8-13 µm thick; sporangium 36-55µm diam, sporangiospores cylindrical to oblong, 3-7 x 3-5µm in size.

***Distribution*:** Commonest soil fungi and the most frequent representative of the mucorales. It occurs in most varied soils including grasslands and forest soils (Borut and Johanson, 1962).

22. *Myrothecium roridum* Tode ex Steudel

Colonies on PDA white, floccose, sporulation after two weeks; reverse yellow, conidiophores closely interwoven, repeatedly branched forming 2-5 branches at each node; phialides hyaline, 13-17 x 1.5-2µm; phialospores rod shaped, narrowly ellipsoid, mostly with both ends truncate, hyaline to dilute olivaceous 6-8.5 x 1.5-2µm.

***Distribution*:** It has been found in forest soils, grass lands, and cultivated soils (Bhatt, 1970).

23. *Oidiodendron* sp.

Colonies on PDA slow growing, grey, slightly floccose, reverse grey; conidiophores pigmented commonly exceeding 50 µm; conidia without a thick walled ring; colonies have no exudation of a dark pigment; pigmented chlamydospores absent, conidia barrel shaped , smooth, 4--6 x 2.5-3µm.

***Distribution*:** A common inhabitant of the humus layers in forest soils (Ellis, 1971).

24. *Penicillium brevicompactum* Dierckx

Colonies 20 mm diam in MEA, radially sulcate, velvety, dark green, exudation absent, reverse greyish green; conidiophores borne from surface mycelium, stipes usually long, smooth walled, characteristically bearing compact, broad terverticilliate penicilli; phialides in divergent verticils of 3-6, ampulliform, 8-10x2-3µm with slightly elongated collula; conidia spherical to subspherical 3-4 x 2.5- 3.5µm with walls roughened. ***Distribution*****:** Relatively uncommon, occupying habitats in soil and decaying vegetation in many geographical areas (Pitt, 1985).

25. *Penicillium chermesinum* Biourge

Colonies in MEA 38 mm diam, deeply sulcate, no exudation present; conidiophores borne profusely from aerial hyphae, stipes stout, smooth walled, strictly monoverticillate, vesiculate; phialides closely packed, ampulliform, relatively short and narrow 7-8 x 1.5-2µm; conidia small, spherical to sub spherical, 2-3µm in size.

Distribution**:** This species has been rarely isolated from India but from a variety of sources (Pitt, 1979).

26. *Penicillium corylophilum* Dierckx

Colonies 40 mm diam in MEA, velutinous; conidiogenesis moderate, conidiophores borne from subsurface hyphae; stipes smooth walled; phialides in verticils of 3-8, ampulliform, 7-12 x 2-2.5µm, with short collula; conidia spheroidal to subspheroidal 2-3.5µm diam, smooth walled.

Distribution**:** This is a widely distributed species and has been isolated from a wide variety of substrates. It does not appear to be of such common occurrence.

27. *Penicillium crustosum* Thom

Colonies 38 mm diam in MEA, with surface fasciculate; conidiogenesis moderate; conidiophores mostly borne from subsurface hyphae, terverticillate; phialides 3-4 per metulae, ampulliform 8-11x 2-2.5µm with short thick collula; conidia spherical 2-4µm diam, with smooth walls.

Distribution**:** it is a common biodegrading fungi, rarely reported from soil (Pitt, 1985).

28. *Penicillium decumbens* Thom

Colonies 49 mm diam MEA, velvetty, conidiogenesis light, in colour, reverse olive brown; conidiophores borne from aerial hyphae, stipes short 38-110µm with thin, smooth walls, penicilli monoverticillate or with limited numbers of irregularly disposed metulae; phialides 4-7per verticil, ampulliform, 7-9 x 2-3µm, with thick collula; conidia subspherical, smooth walled, 2-3.5µm diam.

***Distribution*:** This is a ubiquitous fungus found in soils, decaying vegetation and foods. Reported from USSR, Egypt and Australia (Pitt, 1985).

29. *Penicillium puberulum* Bain.

Colonies 26mm diam in MEA, radially sulcate, surface velutinous to fasciculate; conidiogenesis moderate, dark turquoise, exudate and soluble pigment absent; reverse greyish yellow; conidiophores borne singly from subsurface hyphae, bearing terminal terverticillate penicilli; phialides 3-6 per metulae, ampulliform, 7-9 x 2-3µm with short collula; conidia spherical to subspherical, 2-4µm diam, smooth walled.

Distribution: This has been isolated relatively rarely, although it is widespread (Pitt, 1979).

30. *Penicillium spinulosum* Thom

Colonies 37 mm diam in MEA, radially sulcate, velvety, colour similar to that of conidiophores borne from surface mycelium; stipes long 120-200µm, thin walled, slightly rough, typically monoverticillate, vesiculate; phialides 8-10 per verticil, ampulliform, 8-9x1.5-3µm with short thick collula; conidia spherical with walls spinulose, commonly 2.5-3.5µm diam. (Fig. 5).

***Distribution*:** Common occurrence in soils, decaying vegetation, foods and textiles (Pitt, 1985).

31. *Pestalotiopsis uvicola*

Colony on P.A, 8.2 m diam, straw or pale coloured; hyphae hyaline, smooth walled, branched, septate, 1.8-3.6µm wide; conidiogenus cells colourless, smooth walled, ampuliform; conidia fusiform, slightly curved, 4 septate, 19.8-27.9 x 4.5-6.3 µm size; appendages in two tiers, attenuate, generally 2-4; apical cell acute, colourless; basal cell acute, colurless with single attenuate appendage; median cells short, cylindrical, pale brown and concolourous, septa and periclinal walls darker than the rest of the area; walls smooth with constrictions at the septa; 14 -18.9 µm long (second cell from the base 4.5-5.4 µm long, third cell 4.5- 6.3µm long, fourth cell 4.5-6.3µm long).

***Distribution*:** The known distribution is only from Italy (Nagaraj, 1993).

32. *Phoma glomerata* (Corda) Wollenw. & Hochapfel

Colonies on PDA black, slightly floccose at young stage, reverse black; chlamydospores catenate, conidia produced in pycnidia, and formed from ampuliform phialides, straight cylindrical, slightly tapered towards the base, 4-7-x 2-3µm in size.

***Distribution*:** This fungus is frequently reported from cultivated soils of Israel, Peru, Canada, USA and India (Sutton, 1980).

33. *Sesquicillium candelabrum* (Bonord.) W. Gams

Colonies on PDA white, granulose, reverse brownish yellow; conidiophores branched, bearing phialides of two types, terminal flask shaped supported by a cell with a short lateral phialidic opening; conidia ellipsoidal, 4.5-6 x 2-3 µm in size.

***Distribution*:** This has been frequently isolated from forest and cultivated soils (Gams, 1968).

34. *Staphylotrichum coccosporum* J. Meyer & Nicot

Colonies on PDA slow growing, brown, velvety, reverse dark brown, producing erect conidiophores, brown at the base lighter towards the tip, 410-720µm long, conidiophores with apical recemose branching and solitary terminal globose to subglobose aleuroconidia; conidia globose, 10-16µm in size.

***Distribution*:** This is a widespread soil fungus occurs commonly in warmer climates. It

was originally reported from rainforest soil in Zaire. Later it was reported from Poland, Italy, USA, Africa and grass lands in India (Ellis, 1971).

35. *Torula herbarum* Pers. ex S. F. Gray

Colonies on PDA dark brown, floccose, reverse black; conidiophores smooth, proximal sterile part brown; conidia cylidrical with rounded ends, brown, smooth, 1-5 septate, strongly constricted at the septum, mostly 3-4 septate, 10-24 x 6-8µm in size.

***Distribution*:** It is reported from Netherlands, Italy, Nigeria, India and Japan (Ellis, 1971).

36. *Trichoderma harzianum* Rifai

Colonies on PDA grow rapidly, white turning to green, reverse uncoloured; hyphae septate, branched, smooth walled, hyaline; intercallary and terminal chlamydospores mostly globose; lateral phialides arise in false verticils, short, measuring 6.3-8.1 x 1.8-2.3µm; phialospores produced singly, sub globose, smooth walled, 2.7-3.6µm in size.

Distribution: Worldwide occurrence. It has been isolated from Italy, Libya, Africa (Danielson and Davey, 1973).

37. *Verticillium catenulatum* (Kamyschko ex. Barron & Onions) W. Gams.

Colonies on PDA white floccose, reverse pale yellow; dictyochlamydospores present, produced in aerial mycelium on slender stalks consisting of cluster of several thick walled cells, 21-26 x 17-21µm; verticillate type of conidiophores having long phialides, 11-18 x 1-1.5µm; conidia oval, catenulate, 2-3µm. in size.

***Distribution*:** This is worldwide in distribution in various types of soil

38. *Verticillium fungicola* ?

Colonies on PDA white floccose, reverse white; conidia straight; 4-7 x 3µm; phialides 21-28 µm long; well differentiated conidiophores present with globose conidial heads; dictyochlamydospores absent.

39. *Verticillium lamellicola*?

Colonies on PDA white, slightly floccose; reverse light yellow; conidiophores thin, hyaline, smooth walled, very long; phialides formed in 3-7 verticils, long slender, 15-21x 1-1.5µm; conidia produced in globose heads at the tip of phialides, fusiform to cylindrical, hyaline, one celled 3-6.5 x 1-2µm and smooth walled. (Fig.3).

40. *Verticillium tricorpus*?

Clolnies on PDA slow growing, white floccose aerial mycelium, changing to yellow; reverse yellowish brown, dark brown at centre, producing yellowish colour to the growing medium; conidiophores hyaline; phialides formed in 3-4 verticils; 12-22 x 2-3µm: conidia ellipsoidal to fusiform, hyaline, catenate, 5-7 x 2.5µm in size (Fig.8).

41. *Wardomyces ovalis*

Colonies on PDA dark brown reverse black; conidiophores short with short phialides; conidia one celled, sub hyaline to pale brown, barrel shaped, formed in chains, 5-6 x 3µm in size.

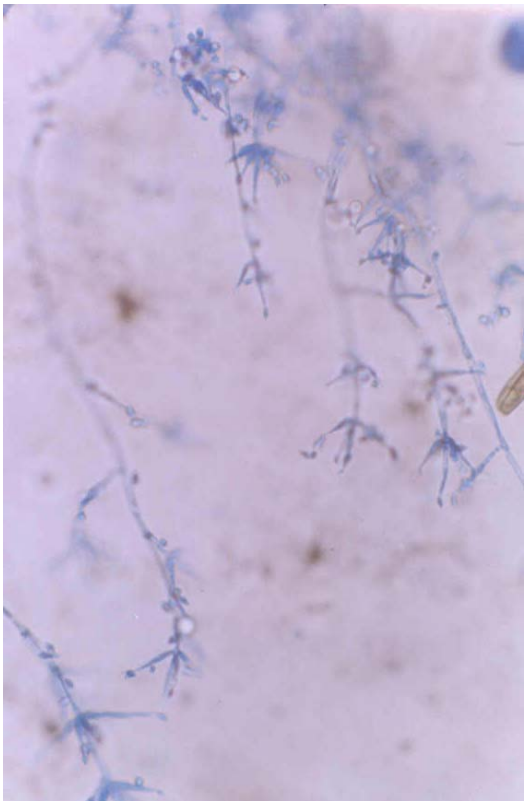

Fig. 3 *Verticillium* sp.

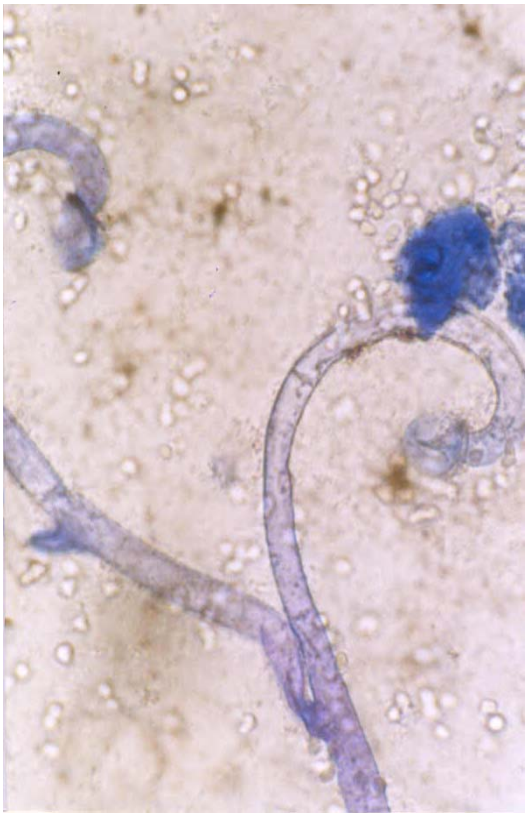

Fig. 4 *Circinella* sp.

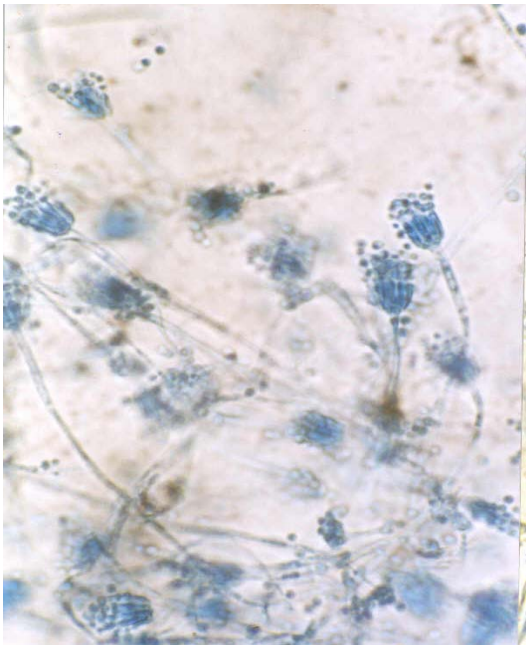

Fig. 5 *Penicillium spinulosum*

Fig. 6 *Aspergillus* sp.

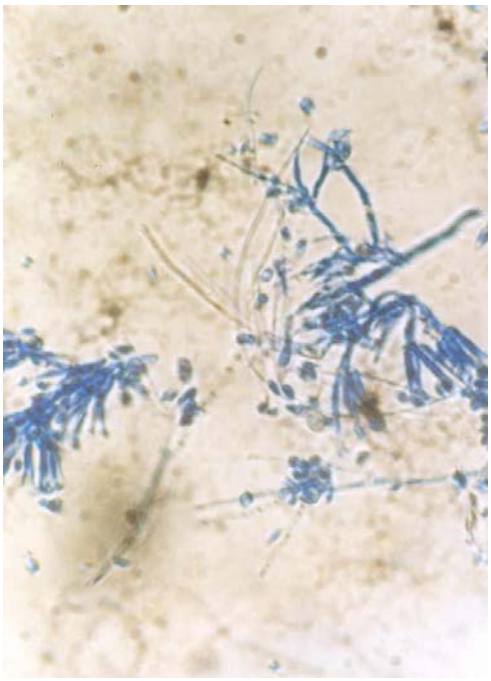

Fig. 7 *Gliocladium roseum*

Fig. 8 *Verticillium* sp.

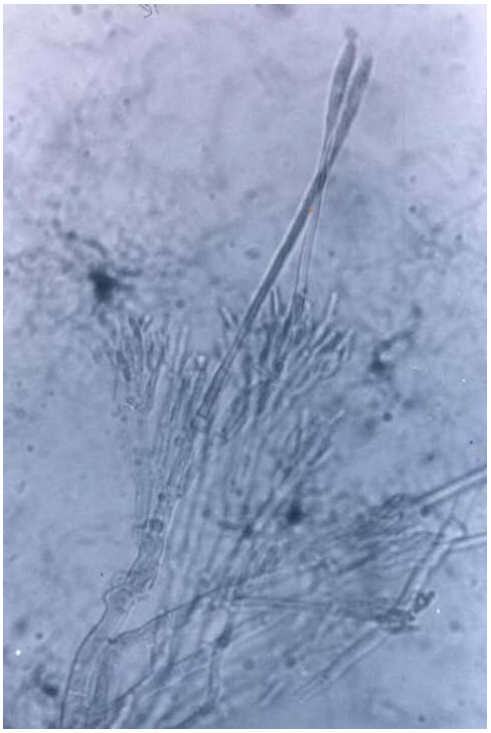

Fig. 9 *Cylindrocladium* sp.

Among the total fungi identified, seventeen fungi are reported as new records to Kerala. (Table 8).

Table 8. The fungi reported as new record to Kerala

Name of fungi	Name of shola
Acremonium fusidioides	Pambadam
Colletotrichum acutatum	Mavannan
Doratomyces stemonites	Meppadi
Fusarium sambucinum	Pambadam
F. stilboides	"
Penicillium brevicompactum	Manthan
P. crustosum	"
P. puberulum	"
P. spinulosum	"
Pestalotiopsis uvicola	Pambadam
Phoma glomerata	Manthan
Rhizopus stolonifer (group)	"
Sesquicillium candelabrum	Meppadi
Verticillium catenulatum	"
Verticillium fungicola	Pambadam
Verticillium tricorpus	Meppadi
Wardomyces ovalis	"

4. CONCLUSION

The study revealed the richness of microbes in the soil as well as leaf litter in the shola forests of the study area. A wide variety of fungi are growing in this unique system of forest. Penicillia, Aspergilli, *Verticillium* and *Trichoderma* dominated the isolations and they are worldwide in distribution irrespective of difference in climatic or edaphic factors. The soil fungal flora of shola forests showed close resemblance to soil mycoflora of temperate region. The abundance of *Penicillium* species in the soil supports this observation. The species richness of soil fungi in the shola forests indicates its importance for biodiversity conservation. Also, the presence of rare species of Penicillia indicates its potential for innovative biotechnology. Seventeen species of fungi isolated during the study are new records from Kerala and among these few species are rarely reported from India.

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Appendix

Potato Dextrose agar (PDA)

Agar	17.00g
Potato (peeled and sliced)	200.00g
Dextrose	20.00g
Distilled water	1000ml

Starch Casein Agar (SCA)

Agar	18.00g
Starch	10.00g
Casein	0.30g
KNO_3	2.00g
NaCl	2.00g
K_2HPO_4	2.00g
$MgSO_4$	0.50g
$CaCO_3$	0.002g
$FeSO_4 .7H_2O$	0.001g
Distilled water	1000ml

Potato Carrot Agar (PCA)

Potato	20.00g
Carrot	20.00g
Agar	20.00g
Tap water	1000ml

Malt Extract Agar (MEA)

Malt extract	20.00g
Peptone	1.00g
Glucose	20.00g
Agar	15.00g
Distilled water	1000ml

25% Glycerol Nitrate Agar (G25N)

K_2HPO_4	0.75g
Czapek concentrate	7.50ml
Yeast autolysate Extract	3.70g
Glycerol analytical grade	250ml
Agar	12.00g
Distilled water	750ml

Czapek Yeast Autolysate Agar (CYA)

Czapek concentrate	10ml
K_2HPO_4	1.00g
Yeast extract	5.00g
Sucrose	30.00g
Agar	15.00g
Distilled water	1000ml

Rose Bengal Agar (RBA)

	20.0 g
Agar	
KH2P04	1.0 g
MgS04 7h20	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Rose bengal (1%)	3.3 ml
Distilled H20	1000.0 ml
Streptomycin	30.0 mg

Preparation: All the materials except rose bengal and streptomycin are dissolved in water. The mixture is heated slowly while stirring until it starts to boil. It is removed from heat and rose bengal is added. After bottling and autoclaving and before pouring plates, streptomycin is added to the cooled liquid medium.

Soil Extract Agar (SEA)

Agar	15.0 g
Glucose	1.0 g
K2HP04	0.5 g
Soil extract	100.0 ml
Tap water	900.0 ml
pH	6.8-7.0

Preparation: Soil extract is prepared by heating 1000 g of garden soil with 1000 ml of tap water in an autoclave for 30 min. About 0.5 g of calcium carbonate is added and the soil suspension is filtered through double paper filters until clear. The extract may be bottled and sterilized in 100 ml quantities.