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Mycodiversity of Orchha Wildlife Sanctuary (M.P.) India

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ABSTRACT

Fungi being saprophytic, play a vital role in decomposition and recycling of litter and forest debries. Fungi also help to keep soil healthy by releasing nutrients.. The objective of current study is to investigate the fungal diversification in Orchha Wildlife Sanctuary, Madhya Pradesh. Samples of degrading litter were collected throughout the year in the Orchha region. The collected material was dissolved in double distilled water and serially diluted upto three times. The Potato Dextrose Agar (PDA) medium-filled petriplates were streaked with both the undiluted and diluted solutions. As slants, pure cultures were kept at 4°C. Macroscopic and microscopic both methods were used to identify fungus isolates. There were 33 distinct fungi that were isolated and named - *Acremonium implicatum, Alternaria alternata, Ascochyta graminicola, Aspergillus flavipes, A. flavus, A. fischeri, A. fumigatus, A. japonicus, A. nidulans, A. niger, A. terreus, A. tamarii, Beltraniella humicola, Ceratocystis paradoxa, Chaetomium salami, Cladosporium sphaereospermum, Colletotrichum capsici, Cunninghamella blakesleeana, Curvularia lunata, Emericella nidulans, Fusarium oxysporum, Gibberella zeae, Humicola fuscoatra, Mucor hiemalis, Paecilomyces lilacinus, P. variotii, Pestalotiopsis mangiferae, Penicillium chrysogenum, P. decumbens, Rhizopus stolonifer, Rhodotorula glutinis, Scopulariopsis brumptii and Trichoderma viride*.

Figures : 33 References : 25 Table : 01
KEY WORDS : Decomposition, Fungi, Litter, Potato Dextrose Agar.

Introduction

In forest soils, plant litter is a significant source of organic carbon. Its decomposition is a complicated process that combines mineralization and change in organic matter. A crucial stage in recycling nutrients is the decomposition of plant litter[3]. The fungus in the soil is essential to the nutrient cycles in forest environments. Saprophytic fungus can decompose soil organic matter and release carbon into the soil, but symbiotic fungi can create symbiosis with plants and benefit each other through nutrient flow.

Fungi are a category of microorganisms that are abundantly present in the environment, particularly in soil[4]. They exist in nature in a saprophytic state because they produce a wide range of hydrolytic enzymes[20]. It is widely known that after insects, fungi make up the second-largest group of organisms on earth[11]. They make upto 90% of all living biomass in forest soils[9], and account for one-third of the microbial population in many forests[7]. Fungi and bacteria both are the decomposers of litter in many habitats but fungi grow as a branching network of hyphae, in contrast to bacteria, which are unicellular organisms. Fungi may use their hyphae to penetrate larger sections of organic matter below the surface, whereas bacteria are only able to grow and feed on the exposed surfaces of organic materials. Fungi break down organic matter by producing enzymes, which they then use to digest the decomposing material and extract the nutrients.

In order to sustain soil fertility and productivity, soil fungus plays a significant and essential function. They are affected by a variety of factors, such as soil characteristics and anthropogenic activities.[1] They are crucial in the nutrition and procedures that improve the health and development of the plant[18]. Although the actual number of fungi is still unknown, only 5–13% of the estimated fungal species around the world have been characterized[16]. As a result, the identification and isolation of fungi from various environmental sources is still crucial for observing and identifying more species, revising scientific classification, assessing their effects on nature, and providing strains for biological control, ecological remediation and industrial aspects[5]. The goal of the current work is to identify the fungi from litter decomposing soil collected from Orchha Wildlife Sanctuary, Madhya Pradesh.

Material and Methods

Collection of samples

The samples of decayed litter were taken from the Orchha Wildlife Sanctuary in Madhya Pradesh (25° 21' 6.91" N and 78° 38' 25.19" E). Freshly decomposing leaf litter was collected in the month of January and October, 2017. They were carried in plastic bags to the lab in order to isolate fungus.

Isolation of fungi

The dilution plate method was used to isolate soil fungus from the collected soil samples[12,25]. To make a litter suspension, 1.0 g of sample was suspended in 10.0 ml, sterile distilled water and shaken for 2-3 minutes at 50 rpm and its dilutions were made up to 10^{-3}. 1.0 ml of the dilution was streaked over agar plates having Potato Dextrose Agar (PDA) medium contained (g/L): Potato – 200.0, dextrose – 20.0, agar – 15.0, pH - 5.6. Inoculated petriplates were incubated at 30°C for fungal growth. Fungal cultures were purified by repeated subcultures. The pure cultures were maintained at 4°C as PDA slants for further work.

Identification of fungi

Pure cultures of fungal isolates were identified based on cultural and morphological characteristics using consult keys from standard texts of mycology[6,10,13,17,19]. Fungal colonies appeared over the medium and they were examined morphologically as well as microscopically.

The morphological characteristics included growth of colony (length and width), presence and absence of aerial mycelium, color of the colony, and spore pigmentation. The mature colonies were stained with lactophenol cotton blue and examined under a microscope. Microscopic analysis included spore structure, conidia, conidiophores, and hyphae structure.

Microscopic Analysis of Fungal Spores

The spore size of the microfungi was measured using a stage and an ocular micrometer. The oculometer was calibrated with a stage micrometer before measuring spore size (µm). The calibration procedure for the ocular micrometer necessitates superimposing the graduations on both micrometers. On the stage micrometer, the number of ocular divisions per known distance was then determined. Finally, for one ocular division, the calibration factor was calculated as follows[15]:

$$\text{One division on ocular micrometer (µm)} = \frac{\text{Known distance between two lines on satge micrometer)}}{\text{Number of divisions on ocular micrometer}}$$

Results and Discussion

Forests that serve as carbon storage and sequestration have a significant role in the global nutrient cycle, in which fungi play an active role. Fungi are one of the predominant groups found in soil and have a significant impact on ecosystem structure and function, providing a critical component for many ecological services. As a result, evaluating soil biodiversity and its biological function is becoming more and more important[2]. Extracellular enzyme activity is affected by the amount and the type of organic materials at the ecosystem level[22].

In this work, samples of decaying litter were collected from several parts of the Orchha Wildlife Sanctuary, M.P. and serially diluted to isolate fungi. The isolated fungi were examined on the basis of cultural, morphological and microscopic characteristics. Fig 01 (1-33) showing thirty three fungal species were isolated and identified. Standard texts and photographs were used for identification. The measurements of cell dimensions were made using a stage and an ocular micrometer as shown in Table-1.

The following are the fungal isolates with their distinguishing characteristics:

1. ***Acremonium implicatum***

   Colonies on PDA moderately growing in 3-5 days, floccose, white at first becoming pale pink at maturity, reverse pale, conidiophores short, simple, erect, smooth and arising from aerial hyphae, conidia one celled, fusiform, smooth, hyaline, produced in very long chains which become tangled in age.

2. ***Alternaria alternata***

   Colonies growing on PDA in 3-5 days, olive black without aerial mycelium, conidiophores short, straight, conidia forming often in long branched chains of 2-10 or more, 3-8 transverse septa, walls rough in lower part with longitudinal or oblique septa, obclavate, ovoid, ellipsoidal, short or cylindrical beak, smooth, golden brown, beak pale.

3. ***Ascochyta graminicola***

   Colonies spreading on PDA in 4-6 days, dark brown, hyphae brown, conidiophores absent, pycnidia gregarious, globose, ostiolate, dark brown, conidia two celled, hyaline, smooth, ovoid to oblong, cylindrical to irregular.

TABLE -1 : Fungal isolates with their dimensions

Fungal Isolates	Length (µm)	Width (µm)
Acremonium implicatum	4-5	1-2
Alternaria alternata	22-30	8-11
Ascochyta graminicola	190-200	180-190
Aspergillus flavipes	2-3	-
Aspergillus flavus	4-6	-
Aspergillus fischeri	1-2	-
Aspergillus fumigatus	2-4	-
Aspergillus japonicus	3-4	-
Aspergillus nidulans	2-3	-
Aspergillus niger	3-4	-
Aspergillus terreus	1-2	-
Aspergillus tamarii	4-5	-
Beltraniella humicola	15-17	5-8
Ceratocystis paradoxa	7-9	2-4
Chaetomium salami	5-6	2
Cladosporium sphaerospermum	3-4	-
Colletotrichum capsici	9-10	6-8
Cunninghamella blakesleeana	30-40	
Curvularia lunata	12-18	8-9
Emericella nidulans	2-3	1
Fusarium oxysporum	7-9	2-3
Gibberella zeae	10-15	3-4
Humicola fuscoatra	8-12	-
Mucor hiemalis	6-8	3-4
Paecilomyces lilacinus	2-3	2
Paecilomyces variotii	3-4	2
Pestalotiopsis mangiferae	18-25	4-6
Penicillium chrysogenum	3-4	2
Penicillium decumbens	2-3	2
Rhizopus stolonifer	8-10	4-6
Scopulariopsis brumptii	4-6	2-4
Trichoderma viride	3-4	2-3

4. ***Aspergillus fischeri***

Colonies on PDA growing in 3-5 days, presenting a dissected appearance, characterized by abundant cleistothecia which are near cartridge buff to light buff; reverse color to pinkish; conidial heads columnar to globose; conidiophores variable in length; vesicle flask shaped, bearing phialides half of the vesicle; phialides uniseriate, crowded, dull green; conidia delicately roughened, globose.

5. ***Aspergillus flavipes***

Colonies growing on PDA in 3-5 days, white at first, turning to bright wheat color later, velvety, reverse usually red brown; exudates usually abundant; conidial heads mostly columnar, conidiophores smooth, yellow to light brown; vesicles subglobose to vertically elongate, biseriate phialides; conidia subglobose and smooth.

6. ***Aspergillus flavus***

Colonies on PDA growing rapidly in 3-4 days, dark yellow green color, reverse colorless to pale yellow brown; conidial heads radiate, splitting into poorly defined columns, conidiophores arising separately from the substratum, roughened, vesicle subglobose; phialides uniseriate and biseriate; conidia globose to subglobose, conspicuously echinulate, yellowish green.

7. ***Aspergillus fumigatus***

Colonies on PDA spreading dull blue-green, velvety to floccose, white at first become colorless to varying in shades; conidial heads columnar, compact, often densely crowded; conidiophores short, smooth, septate, light green, flask shaped vesicle; vesicle bearing single series of closely packed phialides; conidia globose to subglobose, green in mass, echinulate ; sclerotia and cleistothecia absent.

8. ***Aspergillus japonicus***

Colonies on PDA spreading rapidly in 3-4 days, producing purple brown-black conidial heads; white to cream colored colony in centre; reverse purple colored; conidial heads variable, small, radiate or split into indistinct columns; conidiophores arising from the substratum, colorless walls; vesicle globose to elongate; phialides uniseriate; conidia mostly globose, strongly echinulate, purplish brown.

9. ***Aspergillus nidulans***

Colonies on PDA 3-5 days, dark cress green; pinkish cinnamon conidial heads, abundant cleistothecia; reverse purplish red to very dark in age; conidial heads slightly larger; cleistothecia present, hülle cells thick walled, globose to subglobose; asci subglobose to ovate; ascospores red- orange in color; conidiophores light brown; vesicle hemispherical, brown, biseriate phialides; conidia globose to subglobose.

10. ***Aspergillus niger***

Colonies growing on PDA in 3-5 days with abundant sub-merged mycelium; reverse colorless to pale yellow; conidial heads carbon black and large, at first globose then radiate or splitting in well defined columns in age; conidiophores arising directly from the substratum, thick walled; vesicle globose, bearing two series of fully packed phialides, brownish; conidia globose, spinulate, at first cream to buff, later vinaceous buff in age.

11. ***Aspergillus tamarii***

Colonies growing on PDA in 3-5 days, olive brown in young age turning to brownish green or brown with age; reverse of the colony colorless; vegetative hyphae submerged, dull yellow to brown shades in old colonies; conidial heads globose to loosely radiate; conidiophores arising from sub-merged hyphae, rough; vesicle globose; phialides biseriate; conidia globose to subglobose and brown in color.

12. ***Aspergillus terreus***

Colonies growing on PDA in 3-5 days, cinnamon-buff to wood brown, velvety; reverse dull brown; conidial heads long, columnar, compact with cinnamon brown color; conidiophores smooth; vesicle hemispherical, dome shaped, phialides biseriate; metulae crowded parallel; phialides closely packed; conidia globose to subglobose.

13. ***Beltraniella humicola***

Colonies growing on PDA in 5-7 days, effuse, grayish, olive to blackish brown, velvety; setae straight or curved, brown, smooth; conidiophores pale brown, separating cells fusiform; conidiogenous cells polyblastic, denticulate; conidia turbinate, pale to mid brown with hyaline transverse band above the middle.

14. ***Ceratocystis paradoxa***

Colonies dark blackish brown to black; conidiophores colorless to pale brown; arthroconidia catenate, ellipsoidal or obovoid, mid to very dark brown, smooth, thick walled; phialides long and wide in the broadest part; phialoconidia endogenous, at first cylindrical and colorless, becoming ellipsoidal and pale to mid golden brown, truncate at both ends.

15. ***Chaetomium salami***

Colonies growing well on PDA in 4-6 days with cottony, white aerial mycelium; hyphae hyaline, septate; conidia small, subglobose to ovate, slightly roughened; perithecia subglobose to ovate, pale brown, ostiolate, covered with delicate hairs all round

when young, usually mature perithecia having more hairs towards apex, terminal and lateral hairs often similar and indistinguishable; asci clavate to obovate; ascospores brown, subglobose or limoniform or irregular, smooth with single germ pore.

16. Cladosporium sphaerospermum

Colonies growing on PDA in 4-6 days, olive green, becoming olivaceous brown at maturity, velvety or densely powdery; reverse greenish black; conidiophores macronematous and micronematous, varies in length, producing acropleurogenously below septa, olivaceous brown; ramoconidia elongate; conidia spherical or sub-spherical, olivaceous brown.

17. Colletotrichum capsici

Colonies growing on PDA in 3-5 days, dense whitish to dark-grey aerial mycelium; reverse dark brown, conidial mass pale buff to salmon; sclerotia absent; setae abundant, long, bristle like, septate, dark below, lighter above; conidia falcate, fusiform, apices acute; appressoria abundant, medium-brown, clavate to circular, edge usually entire.

18. Cunninghamella blakesleeana

Colonies growing rapidly on OA in 2-4 days, white to gray; sporangiophores long, simple or regularly verticillately branched, lateral branches of the sporangiophores, vary in length and number; terminal vesicles globose to subglobose; lateral vesicles usually smaller than terminal vesicles; sporangioles hyaline, echinulate or smooth, ovoid, ellipsoidal; zygospores globose, brownish with tuberculae.

19. Curvularia lunata

Colonies on PDA on 4-7 days, dark gray, usually zonate; stromata regularly and abundantly formed in culture; mycelium branched, septate; conidiophores long; conidia elliptic, curved, septa 2-3, middle cells broad and darker than other cells, middle septum not median, smooth.

20. Emericella nidulans

Colonies growing on PDA in 3-5 days, velvety, cress green from abundant conidial heads, changing from deep dull to reddish or purple with formation of abundant cleistothecia; reverse drab to purplish red; exudates lacking; conidiophores light brown, sinous,smooth; vesicle hemispherical, brown, bearing phialides borne on metulae; conidia globose to subglobose; hülle cells thick walled; cleistothecia globose to subglobose, breaking to release ascospores; ascospores purple-red.

21. Fusarium oxysporum

Colonies growing on PDA in 3-5 days, aerial mycelium sparse to floccose, white or peach, usually with purple or violet tinge; conidiophores unbranched or sparsely branched, monophialidic; stroma white, plectenchymatous, smooth, effuse; microconidia usually abundant, oval, kidney shaped; macroconidia 2-5 septate, spindle to fusiform, curved or almost straight, pointed at both ends; chlamydospores mostly terminal, globose, smooth.

22. Gibberella zeae

Colonies growing on PDA in 3-5 days, grayish rose to livid red to crimson, often becoming vinaceous with a brown tinge; conidiophores densely branched along with solitary phialides; phialides doliform; conidia slender, falcate, moderately curved with pointed curved apical cell, basal cell pedicillate; chlamydospores scarce, often completely absent, if produced mostly intercalary and in chains; perithecia dark blue, tuberculate; ascospores 3-septate.

23. Humicola fuscoatra

Colonies growing on PDA in 4-6 days, cottony white at first but gradually turning grayish black; bearing masses of chlamydospores and conidia; chlamydospores white at first becoming golden-brown, subglobose; conidia thin walled, pale golden brown; phialoconidia obovoid.

24. Mucor hiemalis

Colonies on PDA growing in 2-4 days, white later buff; reverse pale yellow; sporangiophores slightly sympodially branched; sporangia globose, brownish-yellow; columellae globose to oval, hyaline, rarely yellowish; sporangiospores oval, elliptical to reniform, hyaline, chlamydospores terminal or intercalary; zygospores blackish brown, covered with stellate spines.

25. Paecilomyces lilacinus

Colonies growing on PDA in 5-7 days, vinaceous shades with the reverse uncolored or vinaceous; conidiophores erect, mostly arising solitary from the horizontal mycelium, yellow, rough walled with densely clustered metulae and phialides; conidia ellipsoidal to fusiform, smooth walled.

26. Paecilomyces variotii

Colonies on PDA appeared in 4-6 days, velvety at first then becoming powdery at maturity, yellowish-brown; reverse uncolored to bluish-green shades; odour sweet, aromatic; chlamydospores borne singly or in short chains, globose, brown;; conidiophores repeatedly verticillate; metulae divergent; phialides irregularly distributed along the fertile hyphae; conidia elliptical, yellowish-brown, smooth walled.

27. Penicillium chrysogenum

Colonies growing on PDA in 3-5 days, grayish

Fungal Species

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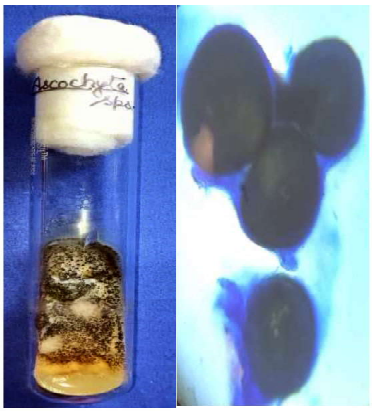

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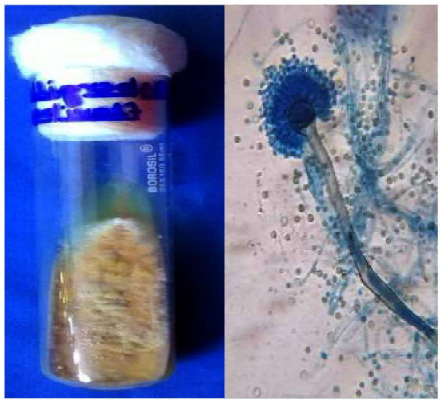

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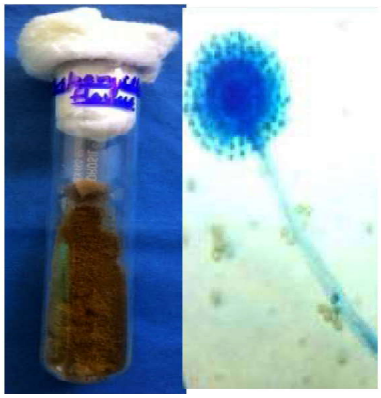

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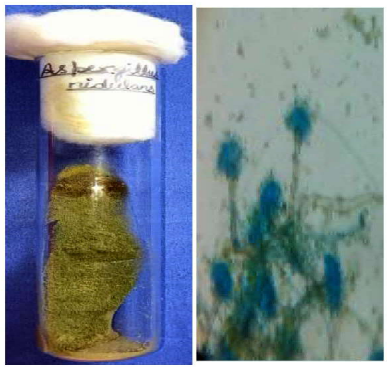

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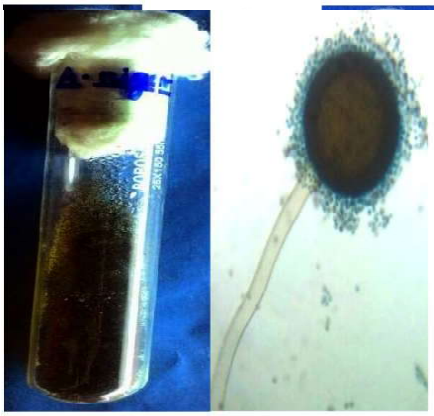

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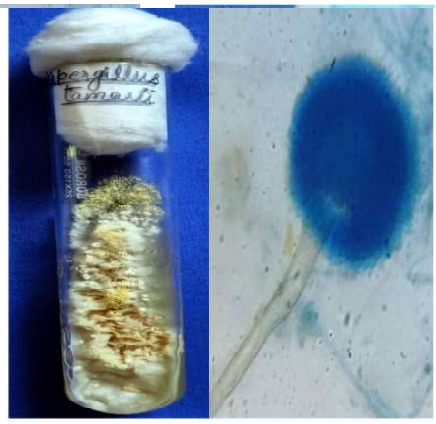

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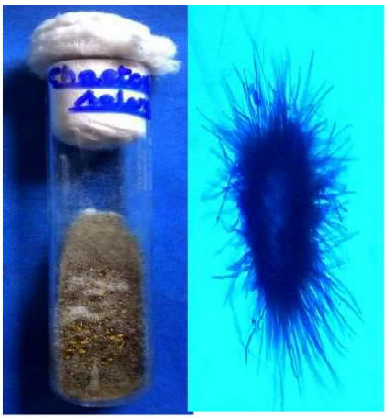

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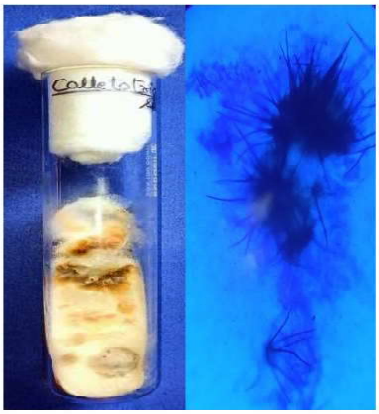

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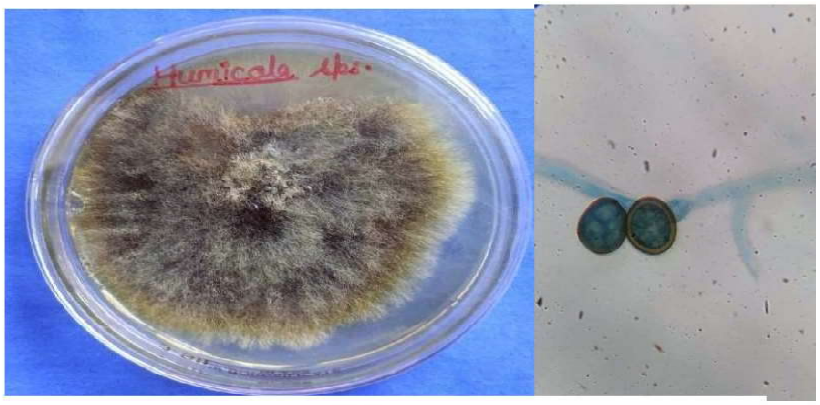

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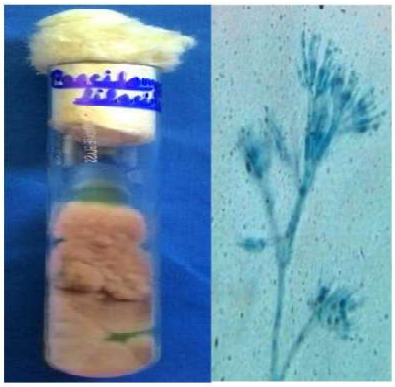

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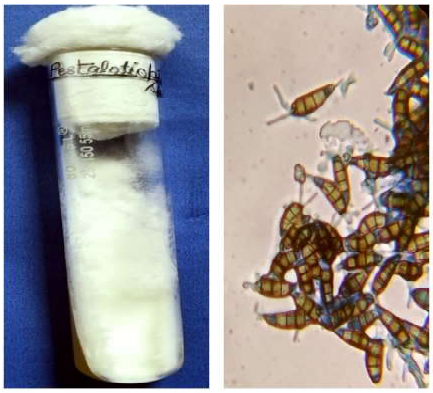

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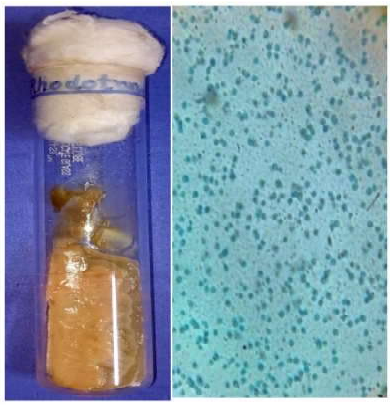

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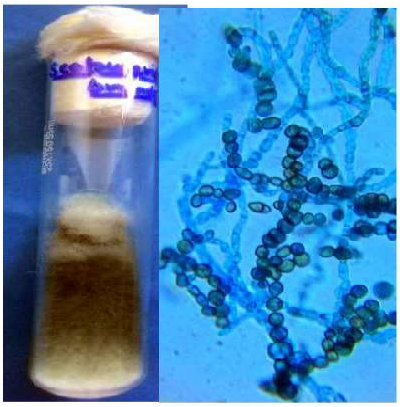

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Figs: 1-33 : Showing fungal isolates with their cultural and microscopic view – (1) ***Acremonium implicatum (2) Alternaria alternata (3) Ascochyta graminicola (4) Aspergillus fischeri (5) Aspergillus flavipes (6)Aspergillus flavus (7)Aspergillus fumigatus (8) Aspergillus japonicus (9) Aspergillus nidulans (10) Aspergillus niger (11)Aspergillus tamarii (12) Aspergillus terreus (13) Beltraniella humicola, (14) Ceratocystis paradoxa, (15) Chaetomium salami,(16) Cladosporium sphaereospermum, (17) Colletotrichum capsici, (18)Cunninghamella blakesleeana, (19) Curvularia lunata,(20) Emericella nidulans, (21) Fusarium oxysporum, (22) Gibberella zeae, (23) Humicola fuscoatra, (24) Mucor hiemalis, (25) Paecilomyces lilacinus, (26) Paecilomyces variotii, (27) Penicillium chrysogenum,(28) Penicillium decumbens, (29)Pestalotiopsis mangiferae, (30) Rhizopus stolonifer, (31) Rhodotorula glutinis, (32) Scopulariopsis brumptii, (33) Trichoderma viride.***

turquoise to dull green or near glaucous blue-green; exudates pale to bright yellow or yellow brown; soluble pigment bright yellow; reverse yellow brown; mycelium white to buff; conidiophores borne from surface, smooth, typically terverticillate, terminal or subterminal, metulae in verticils of 3-5; phialides ampulliform; conidia ellipsoidal smooth.

28. Penicillium decumbens

Colonies growing on PDA in 3-5 days, grayish green to dull green; mycelium white to cream; reverse pale, dull yellow brown or olive, conidiophores borne from aerial hyphae; stipes short, thin walls, smooth, penicillin monoverticillate; phialides 5-8 per vertical, long and slender, ampulliform; conidia ellipsoidal, smooth.

29. Pestalotiopsis mangiferae

Colonies growing on PDA in 5-7 days, blackish brown, acervuli globose to sub-globose; reverse colorless; conidia fusiform, 4 septate with faint constrictions at septa, upper two cells darker, broader, golden brown, apical cell short, conical, hyaline with a 2-3 divergent setulae, tapering, unbranched, basal cell pale golden brown.

30. Rhizopus stolonifer

Colonies growing profusely on PDA in 2-4 days, white at first turning brownish, black, stolons spreading, internodes brown, with well branched brown rhizoides at each node; sporangiophores in clusters, unbranched; sporangia globose, granular, black; columella hemispherical, very often becoming pilate; sporangiospores irregular, round to oval, gray; suspensors swollen; azygospores common; chlamydospores absent.

31. Rhodotorula glutinis

Colonies growing on PDA in 3-5 days, characterized by pink or red, smooth colonies with moist appearance; cells at maturity spherical or ellipsoidal

32. Scopulariopsis brumptii

Colonies growing on PDA in 4-6 days, white at first then turning into grey; reverse smoky grey to fuscous black; hyphae at first hyaline but later darkening; annellophores mostly borne singly, ampulliform; conidia ovoid, truncate at the base, dark brown, smooth walled.

33. Trichoderma viride

Colonies growing on PDA in 4-6 days, watery white becoming hairy from the formation of loose scanty aerial mycelium; conidiation in compact tufts, glaucous to dark bluish green; chlamydospores common, intercalary or terminal; may be formed on the aerial or creeping hyphae, main conidiophores producing smaller side branches, ultimately a conifer like branching system is formed, conidia globose or obovoid.

This work was carried out to identify fungus isolated from degrading litter soil using several morphological and microscopic examination methods. The most important source of fungal isolation is soil. In this study total thirty three fungi were isolated and identified. Ten species from three genera (*Aspergillus, Penicillium* and *Mucor*) were isolated and identified in the rhizosphere soils of diverse agricultural areas in the Nanjangud taluk of the Mysore district, Karnataka, India[8]. This is not surprising given that fungi are saprophytes that feed on rotting plant matter. In contrast to another research[23], the workers isolated fungi from soil contaminated with forest litter, and some researchers isolated fungi from soil, air, and infected plants[21]. The fungi isolated from various sources, including decaying wood, leaf litter, and soil collected from a forest near the Forest Research Institute in Dehradun, were identified as*, Aspergillus niger, A. fumigatus, A. flavus, Fusarium oxysporum*, and species of *Trichoderma, Chaetomium, Curvularia, Penicillium, Alternaria, Trichoderma viride etc.*[14]. In soils, fungi perform a wide range of activities, some of which are active, like decomposing dead plant matter, and others are dormant like propagules that are present in the soil like a resting stage[24].

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