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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND ECOLOGICAL SIGNIFICANCE OF LITTER FUNGI

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ABSTRACT

Fungi are eukaryotic, achlorophyllous, filamentous or unicellular microorganisms. They are ubiquitous and considered the primary decomposers of dead biomass in the biosphere. Decomposition is a key component of global carbon cycling. Fungi play a central role in plant litter decomposition in forest ecosystems through nutrient cycling and humus formation in soil, because they colonize the lignocellulose matrix in litter, which other organisms are unable to decompose. Litter samples were collected from various sites in Salcete, Goa. Macro-fungi maintained in moist chambers and leaf litter were inoculated in sterile petri-plates having PDA media. Colony and microscopic studies of the isolated pure cultures was then done. The litter fungi isolated were *Aspergillus sp., Fusarium sp., Trichoderma sp., Mucor sp., Cheilymenia fimicola, Mycena acicula, Schizophyllum commune, Marasmius haematocephalus and Ganoderma sp.*

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INTRODUCTION

Litter fungi constitute one of the major decomposers communities in the biosphere and are key regulators of nutrients in our ecosystem along with the bacteria and soil fungi (Osono, 2007). Fungi prefer areas with less human interference like forests, grasslands etc. During litter decomposition, inorganic nutrients are released into the environment. The slow cycling of nutrients maintains the stability of the ecosystem.The interaction of litter fungi with the environment (litter) results in decomposing and thereby helps keep our environment clean. They play an important role in decomposition of leaf litter, twigs and wood logs as they contain carbon source which is a regulating factor (Crowther et al., 2012). Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently broken down by cellulolytic fungi, bacteria, actinomycetes and protozoa.Cellulase systems of local fungi should be investigated keeping in view the importance and application of the cellulases (Khokhar, et al., 2012). The major objective of this study is to understand the habit and nature of litter fungi in Salcete Taluka so that further research can be done on cellulase activity of these isolated litter fungi as they are one of the major cellulose producers.

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MATERIALS AND METHODS

Study area and Sample Collection-The Salcete taluka of South Goa district in Goa state was taken up as the study area. Samples such as leaf litter, dead twigs and decomposed bark from terrestrial habitats were temporarily stored in collection/ polythene bags. Isolation and identification of litter fungi-The moist chamber technique (Cannon & Sutton, 2004) was used for isolating fungi from the leaf litter. The litter segments were observed under a microscope every alternate day, for the presence of fungal growth. Also, litter incubation on sterile PDA Petri plates was done in aseptic conditions to facilitate fungal growth. Obtaining Pure Cultures-Pure cultures were obtained from the mixed culture plates. From the Petri plates having mixed culture, desired micro-organisms were transferred to freshly poured petri platescontaining media, using an incinerated needle. This transfer was done in front of the flame in the UV sterilised laminar air flow, in order to avoid entry of contaminants. All precautions were taken to prevent any form of contamination. Preparation of microscopic slides-Preparation of microscopic slides of the isolated fungi, to aid identification was done using the pure cultures. The isolates were identified and assigned to respective genera and species depending on their diagnostic features in the identification of fungi.

RESULTS

The nine litter fungi isolated were *Aspergillus sp., Fusarium sp., Trichoderma sp., Mucor sp., Cheilymenia fimicola, Mycena*

acicula, Schizophyllum commune, Marasmius haematocephalus and Ganoderma sp. Trichoderma spp. showed slightly higher frequency than *Aspergillus* spp. The ubiquitous nature of *Aspergillus* spp. was confirmed by its occurrence at different areas ranging from saline to non-saline, polluted to non-polluted and undisturbed to partially disturbed areas. Similar results about the ubiquitous nature of *Aspergillus* spp.

Aspergillus species

Saprophytic *Aspergillus spp*. found five times in soil and once in litter collected from various areas of Salcete Taluka like Gogol, Margao. Thus, confirming its ubiquitous nature. The colonies were fast growing (Table 1), with smooth or wavy margin (Fig 1). Smoky patches in appearance in petri plates.

Table 1. Colony Study of Identified and successfully cultured Litter Fungi

Name of litter fungi	Sub division	Location of isolation	Shape of the colony	Colour	Size (cm)	Margin	Texture	Growth rate	Reverse Colour
Fusarium Spp.	Ascomycotina	Fatorda	C	White	4.2	R	Dry	Slow	Red
Trichoderma Spp.	Ascomycotina	Fatorda	C	White green circles	5.4	Sm	Dry	Fast	White green circles
Aspergillus spp.	Ascomycotina	Margao	I	White with black spores	8.0	Sm	Dry powdery	Fast	White
*Mucor*spp.	Zygomycotina	Betalbatim	C	White	2.0	Sm	Dry	Slow	White
Schizophyllum commune	Basidiomycotina	Fatorda	C	White	5.8	Sr	Dry, cottony	Slow	White

C- Circular; I- Irregular; R- Rough; Sm-Smooth; Sr- Serrated

Plate 1

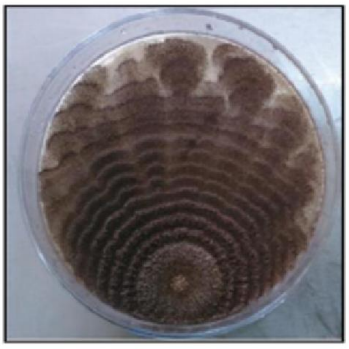

Fig. 1. Colony of *Aspergillus sp.*

Fig. 2. Colony of *Fusarium sp.*

Fig. 3. Colony of *Trichoderma sp.*

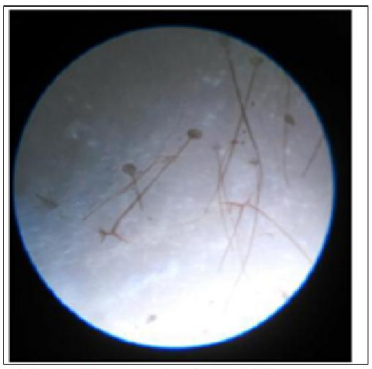

Fig. 4. Microscopic view of *Mucor sp.*

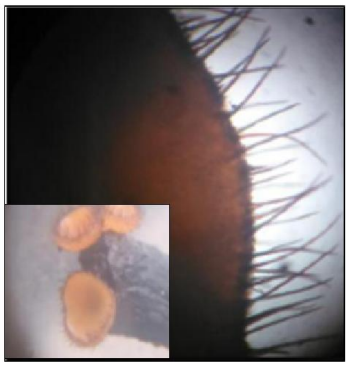

Fig. 5. *Cheilymeniafimicola*- in natural habitat and Microscopic view.

Fig. 6. *Mycena acicula* as seen under dissection microscope

Fig. 7. *Schizophyllum commune* as seen under dissection microscope.

Fig. 8. *Marasmiushaematocephalus*as seen under dissection microscope.

Fig. 9. *Ganodermasp*seen growing on a wood log.

Brown coloured spores with white mycelium. Microscopic observation showed that the mycelium was hyaline, septate and branched. The cells were multi nucleated. Conidia were globose in shape.

***Fusarium* Species**

A litter fungus growing on a twig, collected from Fatorda. Fusarium colonies usually slow growing, pale or brightly coloured may or may not have a cottony aerial mycelium (Table 1). Colony shape circular with rough margin (Fig. 2). Colour of thallus varied from white, orange and purple shades also observed by the scientist of University of Adelaide (2015).

Trichoderma spp

Trichoderma sp. frequently found in soil samples and once in litter sample. Found to be fast growing in petri-plate (Table 1), Colonies greenish in colour, with smooth margin (Fig. 3). Conidiophore highly branched, hyaline, flask shaped and inflated at the base, conidia green in colour, spores found clumped/ grouped, also noted by Dr. Schwarze (2009).

***Mucor* species**

Mucor species was isolated from litter. Microscopic studies of genus *Mucor* showed the absence of stolons and rhizoids. Colonies in pertriplate were seen to be slow growing (Table 1), cottony to fluffy, white to yellow, becoming dark-grey, with development of sporangia. Sporangiophores erect, simple or branched, forming large, terminal, globose to spherical, multispored sporangia, with well-developed subtending columellae (Fig.4). Sporangiospores black (The University of Adelaide, 2015).

Cheilymenia fimicola

*Cheilymenia fimicola*found growing on waste organic matter. Ascomycetes fungus are saprophytic in nature. They are tiny disc like, were small orange cups, cylindrical to cushion shaped becoming shallowly cupulate to saucer shaped, concave, sessile, usually in groups. Fertile upper surface bright orange and smooth and the lower surface slightly paler and covered with fine bristle like hair (**Fig.5**), hair straight, septate, tapered towards the end.

Mycena acicula

Mycena acicula collected from a dead decaying leaf. *Mycena acicula* was identified by using mycokeys. This striking little bonnet mushroom occurs solitary in moist (Fig. 3.1), shaded habits; fruiting on litter, but not attached, also mentioned by Kumm. (2007). Cap about 1cm across; conical, bell shaped; smooth with marginal striations. Reddish when very young (Fig. 6), but soon became mid-orange and a lighter shade of orange towards the rim. Gills adnexed; white with paler gill edges. Stem about 5cm X 2mm, orange shaded, smooth, particularly towards apex, also observed by Pat O'Reilly (2011).

Schizophyllum commune

Schizophyllum commune, "Splitgill", a wood-rotting fungus found growing on dead twig (Fig. 7). Fruiting body was 3-4 cm long, fan shaped when attached to the dead twig and without stem. Upper surface covered with hair, white in colour. Under surface composed of radial gill like folds, each of which was centrally split. Gills function to produce basidiospores on their surface, also mentioned by Volk (2000). The colony showed circular outline with serrated margin as mentioned in Table 1.

Marasmiushematocephalus

Marasmius haematocephalus easily identified because the pileus is coloured in various tinges of red (Fig. 8). Distant lamellae, and brown stipe at base, long and slender basidiospores and well developed pleurocystidia. Pileus 3–15 mm broad, mostly campanulate (shaped like a bell), then convex, sulcate striated (marked with parallel groove), slightly reflexed to uplifted at crenulate margin (notched outline), when oldmembranaceous, pale red. Lamellae distant, free to adnexed, narrow to broad. Stipe filiform (filament or thread like), cylindrical, hollow, glabrous, smooth, lustrous (shiny or glossy), reddish brown to dark brown, paler apex and white tomentose basal mycelium, also observed by Antonin (2006).

Ganoderma species

Ganoderma sp. was collected on a dead decaying wood log (Fig. 9) along with some bryophytes. *Ganoderma* is largest genus having more than 300 species Bhosle (2010). Ganoderma spp. are white-rot fungi with enzymes that allow them to break down wood components such as lignin and cellulose*,* cosmopolitan in distribution. All *Ganoderma* species are polypores with a bright white pore surface that bruises brown when touched or scratched and produce brown spores. Spore deposits are usually found coating the tops of caps at full maturity.

Conclusion

Biotic interaction of fungi with decomposing matter is beneficial to humans as they are indispensable part of natural decomposers and thus keep the environment clean. Litter fungi are considered to be the key players in litter decomposition because of their ability to produce a wide range of extracellular enzymes, which allows them to efficiently attack the recalcitrant lignocellulose matrix that other organisms are unable to decompose. Hence, these emphasize on the essentiality of the litter fungi studied.

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