

## Fungal Diversity on the Leaf Litter of *Syzygium calophyllifolium* Walp.

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### Abstract

The decomposition of plant litters on the soil surface is brought about by a variety of microorganisms including bacteria, fungi and actinomycetes. The aim of the present study was to study the ecology of the colonizing fungi associated with the degradation of leaf litter of *Syzygium calophyllifolium* Walp. The colonization of fungi was analyzed in three different grades of litters (Grade 1-L, Grade 2-F1 layer, Grade 3-F2 layer). In presenting the data, two terms viz. Frequency of occurrence (FOC) and percentage of occurrence (POC) were used throughout the test. Altogether 36 species were isolated from three grades of litter by moist chamber incubation technique. These included 4 zygomycetes, 2 ascomycetes and the remaining 30 belonged to deuteromycetes. An analysis of the data obtained on the three grades of litter suggested that 15 species belonging to 12 genera were recorded from 'Grade 1', 25 species belonging to 19 genera were recorded from 'Grade 2' and 18 species belonging to 15 genera were recorded from 'Grade 3' litter. A comparison of the mycoflora of the three grades reveal that while some are common to all the three grades, a few were found restricted to any two grades and still some to a particular grade.

**Keywords:** *Syzygium calophyllifolium*; *Eugenia calophyllifolia*; *Myrtaceae*; *Decomposition*; *Fungal diversity*; *Litter*

### Introduction

Litter decomposition playing a major role in the transfer of energy and nutrients [1] and is an important process of nutrient cycling in forest ecosystems [2]. The nutrient availability both for plant growth and ecosystem productivity was contributed directly by litter decomposition [3]. Succession is the main concept of establishment of plant ecology and the application of number of studies in fungal ecology. Fungi are the decomposers of plant materials and thus variety of organic constituents are given to the succession of the fungi of the ecological groups, an organism in the organic constituents until the complete decomposition. These organic constituents are the basic for the classification of the fungi into various ecological groups.

Because of the involvement of fungi in nutrient cycling [4,5], their mycorrhizal and endophytic associations with plants [6-8], and their interactions with insects [9,10] fungi are known to be vital contributors to ecosystems. There are numerous ecological microfungi, especially the pattern of fungal colonization on naturally occurring plant debris above the soil. Studies of fungal succession on plant substrates are well documented [11-15]. Different aspects of microbial colonization of aerial plant parts and their interaction on the plant surface were reviewed [16].

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Fungal succession is a time-related change in fungal community structure [17]. In other words, the study of fungal succession involves analysis of the changes in the structure of fungal communities on various substrata over time [18-20]. Most studies on fungal succession have taken a synecological approach, recording species assemblages at different stages of decay [20]. There have been several studies on fungal succession on wood worldwide [21-24], however, only a few have focused on terrestrial wood. The present study was aimed at understanding the pattern of fungal colonization of litter of the Mycoflora of *Syzygium calophyllifolium* Walp. (*Eugenia calophyllifolia* (Walp.) Wt.) a tall, huge tree commonly called Neerodam belongs to the family Myrtaceae.

## **Materials and Methods**

### **Study area**

The Nilgiris District is located between 11°, 12' and 11°, 43' latitude and between 76° 14' and 77° longitude is the meeting place of three important mountain system viz., the Western Ghats, the Eastern Ghats and the Southern Ghats. The different types of vegetation met with are wet evergreen forest, mountain shola forest, moist deciduous forest, dry deciduous forest. Among all this forests, the montane forest or wet evergreen forest are locally called "sholas". The topography and climate are so conducive for the growth and establishment of number of shola trees. Whole these plants were valuable both for state departments as well as individuals in earning much needed revenue.

### **Collection of sample**

The litter samples belong to shola forest tree species of *Syzygium calophyllifolium* Walp. (Myrtaceae) were collected from four roads near Doddabetta, the Nilgiris, Tamilnadu. The general laboratory techniques followed in the course of the present study were those outlined by [25,26]. The litter was sorted out into three Grades representing Grade 1 (L layer), Grade 2 (F1 layer), and Grade 3 (F2 layer) respectively, representative of different stages of decomposition as follow (PLATE 1):

Grade 1(L): Hard, yellowish brown leaves of the uppermost layers that have recently fallen on the ground and do not show clearly any signs of decomposition at all. The leaves lie in a loosely composed layer with leaves having high tensile strength.

Grade 2(F1): Leaves decomposed but still recognizable. They are dark brown to grey, that have become somewhat thinner than the L layer with well-preserved structure but with conspicuous area of moldy growth and are compact below the L leaves. Fungal activity in this layer is intensive (Fermentation layer 1 or F1 layer).

Grade 3(F2): Leaves in advanced stages of decomposition, are usually fibrous, decolorized, fragmentary and in various stages of decomposition (Fermentation layer 2 or F2 layer).

Grade 2 litters are in active phase of decomposition and Grade 3 litters are obviously in the final stage of decomposition.



Grade 1



Grade 2



Grade 3

**PLATE 1: Different grades of litters.**

### **Isolation of fungi from litter**

Fungi were isolated from litter sample by moist chamber incubation technique. Twenty leaves per sample from each grade of litter were randomly selected and incubated in sterile moist chambers in Petri plates of 15 cm diameter. The Petri plates were first sterilized and the chambers were then prepared by placing within them sterilized filter paper discs which were moistened by adding sterile water but the filter paper was never flooded with sterile water. The sterile water was added periodically to maintain the moisture content. The leaves were incubated for a period of 48 hours and then examined under a binocular stereomicroscope for fungi sporulating on them. All the fungi found in sporulating state were isolated, examined and identified down to species level as far as possible. Where necessary, the fungi were sub cultured on Potato Dextrose Agar Slants for further study.

### **Preparation of lacto phenol**

Semi-permanent slides were prepared by using lacto phenol or lacto phenol with cotton blue as mountant. The stain was prepared by mixing phenol crystals (20 gm) in distilled water (20 ml) and warmed in a water bath till it dissolves completely. Lactic acid (20 ml) and glycerol (40 ml) followed by cotton blue (0.05 gm) were added to this.

### **Slide preparation, illustration, and photomicrography**

Plain lacto phenol and lacto phenol with cotton blue stain were used as mounting media for preparing semi-permanent slides which were sealed with DPX mountant (supplied by BDH chemical division, Glaxo Laboratories (India) Ltd., Bombay. The photomicrographs were taken with Getner microscope (India) Nt. No. 14884 with ASAHI PENTAX X 35 mm. Camera Konica 35 mm-125 ASA color negative film was used for photography.

### **Presentation of data**

In presenting the data, two terms viz. frequency of occurrence (FOC) and percentage of occurrence (POC) were used throughout the test. The term frequency is used to denote the number of times a particular fungus was recorded in the entire course of investigation and expressed as a percentage of the total number of samplings made (6 in the present study). Based on the frequency, five categories of fungi were recognized, namely those showing a Percentage frequency of 81-100 'Dominant', 61-80 'Common', 41-60 'Frequent', 21-40 'Occasional' and 1-20 'Rare'.

The term 'Percentage of occurrence' is used to denote the number of leaves on which a particular fungus was present as against 20 leaves examined per layer by moist chamber incubation and is calculated as follows:

$$\frac{\text{Number of leaves on which a fungus is found}}{\text{Number of leaves examined (20)}} \times 100$$

## Results

### Mycoflora of 'Grade 1' litter

A total of fifteen species belonging to twelve genera were recorded on 'Grade 1' litter. Zygomycotina was represented by four species and the remaining eleven species belonged to Deuteromycotina. No Ascomycotina was recorded on 'Grade 1' litter. The total number of species recorded per sampling varied from 3-8. The maximum numbers of species were recorded in the first and third samplings.

Six species namely, *Absidia glauca*, *Rhizopus stolonifer*, *Drechslera hawaiiensis*, *Gliocladium virens*, *Sympodiella laxa*, *Tetraploa aristata* were 'Dominant', two species namely, *Mucor recemosus*, *Curvularia pallescens* were 'Common', six species namely, *Absidia cylidospora*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Penicillium citrinum* were 'Frequent', *Aspergillus niger* was the only 'Occasional' and there was no 'Rare' forms were recorded on 'Grade 1' litter.

### Mycoflora of 'Grade 2' litter

A total of twenty-five species belonging to nineteen genera were recorded on 'Grade 2' litter. Zygomycotina was represented by one species, two species belonging to Ascomycotina and the remaining twenty-two species belonging to Deuteromycotina were recorded on 'Grade 2' litter. The total number of species recorded per sampling varied from 4-9. The maximum numbers of species were recorded in the first and sixth samplings.

Six species namely *Chaetomium biapiculatum*, *Aspergillus niger*, *Dactylaria purpurella*, *Fusarium oxysporum*, *Penicillium implicatum*, *Tetraploa aristata* were 'Dominant', three species namely, *Gyrophthrix circinata*, *Penicillium funiculosum*, *Sympodiella laxa* were 'Common', six species namely, *Absidia cylidospora*, *Emericella nidulans*, *Aureobasidium pullulans*, *Graphium penicillioides*, *Monodictys putredinis*, *Pestalotia palmorum* were 'Frequent', five species namely, *Fusidium griseum*, *Monodictys pelagica*, *Penicillium citrinum*, *Penicillium rubrum*, *Trichoderma koningii* were 'Occasional', five species namely, *Fusarium solani*, *Hyalodendron sp.*, *Nigrospora sphaerica*, *Phialophora richardsiae*, *Trichoderma aviride* were 'Rare' forms were recorded on 'Grade 2' litter.

### Mycoflora of 'Grade 3' litter

A total of eighteen species belonging to fifteen genera were recorded on 'Grade 3' litter. Zygomycotina was represented by two species; two species belonging to Ascomycotina and the remaining fourteen species belonged to Deuteromycotina were recorded on 'Grade 3' litter. The total number of species recorded per sampling varied from 4-6. The maximum numbers of species were recorded in the first and fourth samplings.

Five species namely *Chaetomium biapiculatum*, *Aspergillus niger*, *Penicillium citrinum*, *Tetraploa aristata*, *Phoma herbarum* were 'Dominant', four species namely, *Rhizopus stolonifer*, *Cladosporium cladosporioides*, *Graphium penicillioides*, *Sympodiella laxa* were 'Common', five species namely, *Mucor recemosus*, *Curvularia pallescens*, *Drechslera hawaiiensis*, *Monodictys putredinis*, *Penicillium funiculosum* were 'Frequent', three species namely, *Emericella nidulans*, *Aspergillus fumigatus*, *Trichoderma viride* were 'Occasional' and the only species *Curvularia lunata* was 'Rare' forms were recorded on 'Grade 3' litter.

### Mycoflora of different grades of litter

Altogether 36 species were isolated from the three grades of litter. These included 4 Zygomycetes, 2 Ascomycetes and the remaining 30 belonged to the Deuteromycetes. An analysis of the data obtained on the three grades of litter suggest that 15 species belonging to 12 genera were recorded from 'Grade 1', 25 species belonging to 19 genera were recorded from 'Grade 2' and 18 species belonging to 15 genera were recorded from 'Grade 3' litter. A comparison of the Mycoflora of the three grades revealed that while some are common to all the three grades, a few were found restricted to any two grades and still some to a particular grade, is given in TABLE 1.

TABLE 1: Occurrence of fungi on different grades of litter.

S.NO	Species	Grade 1	Grade 2	Grade 3
1	<i>Absidia glauca</i>	+	-	-
2	<i>Absidia cylidospora</i>	+	+	-
3	<i>Mucor recemosus</i>	+	-	+
4	<i>Rhizopus stolonifer</i>	+	-	+
5	<i>Chaetomium biapiculatum</i>	-	+	+
6	<i>Emericella nidulans</i>	-	+	+
7	<i>Alternaria alternate</i>	+	-	-
8	<i>Aspergillus fumigates</i>	+	-	+
9	<i>Aspergillus niger</i>	+	+	+
10	<i>Aureobasidium pullulans</i>	-	+	-
11	<i>Cladosporium cladosporioides</i>	+	-	+
12	<i>Curvularia lunata</i>	+	-	+
13	<i>Curvularia pallescens</i>	+	-	+
14	<i>Dactylaria purpurella</i>	-	+	-
15	<i>Drechslera hawaiiensis</i>	+	-	+
16	<i>Fusarium solani</i>	-	+	-
17	<i>Fusarium oxysporum</i>	-	+	-

18	<i>Fusidium griseum</i>	-	+	-
19	<i>Gliocladium virens</i>	+	-	-
20	<i>Graphium penicillioides</i>	-	+	+
21	<i>Gyothrix circinata</i>	-	+	-
22	<i>Hyalodendron sp.</i>	-	+	-
23	<i>Monodictys putredinis</i>	-	+	+
24	<i>Monodictys pelagic</i>	-	+	-
25	<i>Nigrospora sphaerica</i>	-	+	-
26	<i>Penicillium citrinum</i>	+	+	+
27	<i>Penicillium funiculosum</i>	-	+	+
28	<i>Penicillium implicatum</i>	-	+	-
29	<i>Penicillium rubrum</i>	-	+	-
30	<i>Phialophora richardsiae</i>	-	+	-
31	<i>Sympodiella laxa</i>	+	+	+
32	<i>Tetraploa aristata</i>	+	+	+
33	<i>Trichoderma viride</i>	-	+	+
34.	<i>Trichoderma koningii</i>	-	+	-
35.	<i>Pestalotia palmorum</i>	-	+	-
36.	<i>Phoma herbarum</i>	-	-	+
(- indicates absence), (+ indicates presence)				

*Aspergillus niger*, *Penicillium citrinum*, *Sympodiella laxa* and *Tetraploa aristata* are common to all the three grades (FIG. 1.) of litters. *Absidia cylindospora* is common only to Grade 1 and Grade 2 litters. *Chaetomium biapiculatum*, *Emericella nidulans*, *Graphium penicillioides*, *Monodictys putredinis*, *Penicillium funiculosum* and *Trichoderma viride* are common to Grade 2 and Grade 3 litters. *Mucor recemosus*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Curvularia pallescens* and *Drechslera hawaiiensis* are common only in Grade 1 and Grade 3.

*Absidia glauca*, *Alternaria alternata* and *Gliocladiumvirens* are recorded only from Grade 1. *Aureobasidium pullulans*, *Dactylaria purpurella*, *Fusarium solani*, *Fusariumoxysporum*, *Fusidium griseum*, *Gyothrix circinata*, *Hyalodendron sp.*, *Monodictys pelagica*, *Nigrospora sphaerica*, *Penicillium implicatum*, *Penicillium rubrum*, *Phialophora richardsiae*, *Trichoderma koningii* and *Pestalotia palmorum* are recorded from Grade 2. *Phoma herbarum* is recorded exclusively from Grade 3 litters.

The frequency of particular fungus is not the same on all the three grades. *Aspergillus niger* was ‘Occasional’ on ‘Grade 1’, ‘Dominant’ on ‘Grade 2’ and ‘Grade 3’. Similarly, *Penicillium citrinum* was ‘Frequent’ on ‘Grade 1’, ‘Occasional’ on ‘Grade 2’ and ‘Dominant’ on ‘Grade 3’. More or less similar observations were reported by earlier workers on the substrata worked out by them.

**Average percentage occurrence of fungus**

A comparison of the number of species recorded on the three grades of litter shows, the number of species recorded were higher on ‘Grade 2’ when compare with ‘Grade 1’ and ‘Grade 3’ litters and is shown in FIG. 2. The extent of development of the different fungi on litter in various grades of decomposition different in the various fungal species. In the present study, the average percentage occurrence of fungus has been taken as an index of its activity on different grades of litter. It was found that on ‘Grade 1’ *Absidia glauca*, *Sympodiella laxa*, *Tetraploa aristata*, *Gliocladium virens*, *Drechslera hawaiiensis*\_were most active in that order. On ‘Grade 2’ litter the species that were most active are *Aspergillus niger*, *Chaetomium biapiculatum*, *Penicillium implicatum*, *Fusarium oxysporum*, *Dactylaria purpurella* in that order. On ‘Grade 3’ the most active species were *Phoma herbarum*, *Chaetomium biapiculatum*, *Penicillium citrinum*, *Aspergillu sniger*, *Tetraploa aristata* in that order.

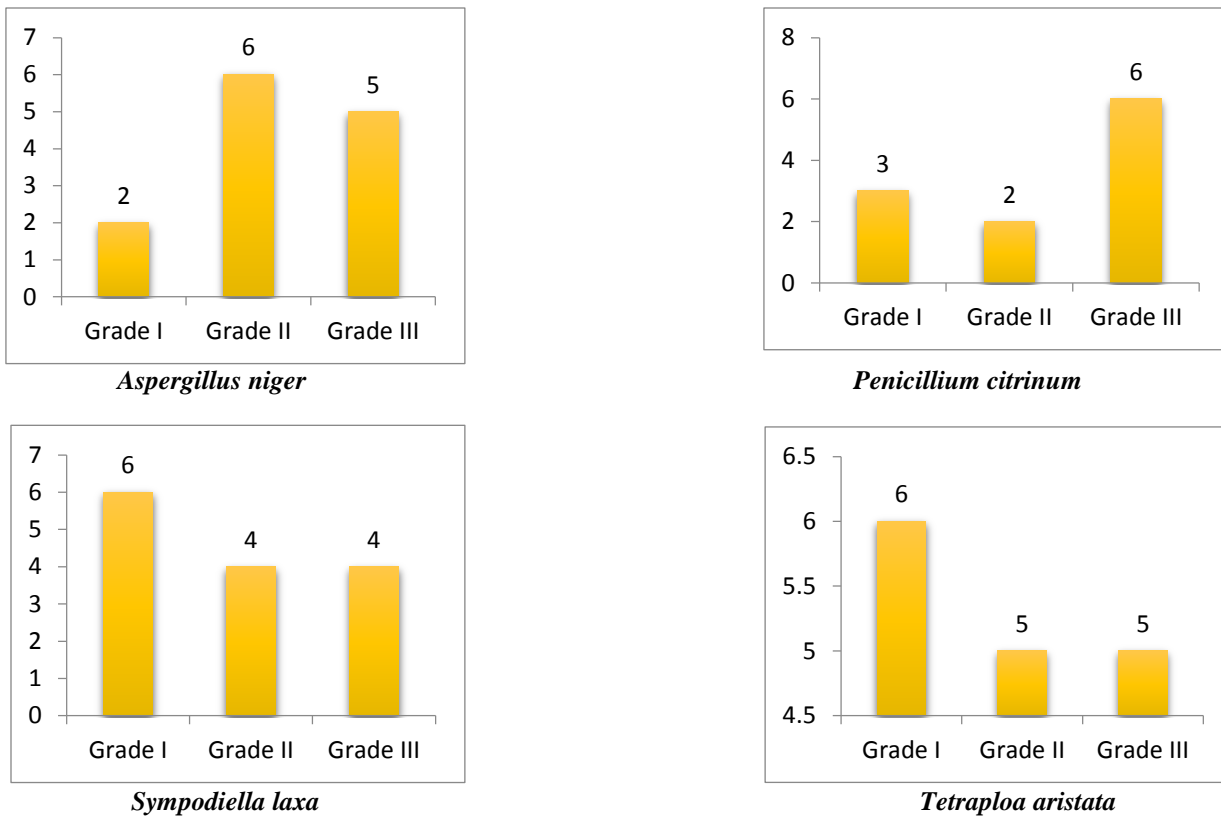


FIG. 1: Species common to all the three grades of litters.

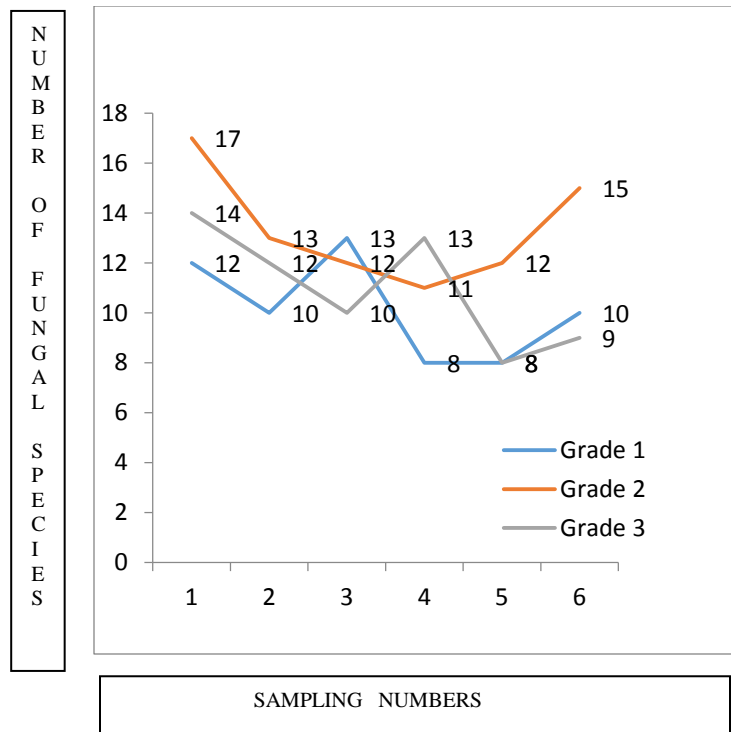


FIG. 2: Comparison of 3 grades of litter- Average percentage occurrence of fungus.

### Discussion

The study of the pattern of fungal colonization on leaf litter using litter bags was demonstrated by [27], who carried out succession of micro fungi in *Phillyrea angustifolia* litter in a Mediterranean maquis in Sardinia. They described two main groups of fungi involved in the process of litter degradation, whose presence was correlated with the successive decomposition stages of the substrate and with seasonal variations. Fungal succession and decomposition on *Shorea obtusa* leaves using polyethylene litter bags to examine the effect of colonization by ligninolytic and cellulolytic fungi in the overall process of decomposition was studied earlier [28].

Different methodologies were carried out to study fungal diversity on the leaf litter in different communities. The methodology of indirect isolation often results in more taxa (including non- sporulating morpho species) than direct observation [29-32]. Indirect isolation methods have been criticized [32] because they recover both active fungi and dormant spores that do not participate in leaf decomposition. Incubating the litter samples in Moist chambers and observing the fungi developing there on still remains the best way of analyzing Mycoflora [33-38], as one can observe many of the typical litter fungi in sporulating condition and which rarely show up in cultural methods. The direct method, in which the substratum is examined in the field or laboratory for fruiting bodies, is regarded as the most common approach for taxonomic purposes [20].

There have been very few studies on the Mycoflora colonizing litter of shola forest trees all over the world and a very little work done in India so far. The main objectives of the present investigation were:



- i) the isolation of fungi from litter samples of *S. calophyllifolium* Walp. a shola forest tree and their identification down to the species level
- ii) to assess the relative survivability and density, distribution, and decay activities in terms of percentage frequency and percentage occurrence respectively
- iii) to get an idea on the pattern of colonization of *S. calophyllifolium* litter.

In the present study litter collected in each sampling was classified into three categories, viz., 'Grade 1', 'Grade 2', and 'Grade 3', representing increasing degree of decomposition judged from the general texture and appearance of the leaves. Although this grading looks arbitrary, a critical examination of the litter, especially the morphology and texture of leaves suggest that the three grades more or less represent litter layer, 'fermentation layer 1 and 2' respectively. The Mycoflora of litter was analyzed by direct observation after moist chamber incubation technique.

### Conclusion

The examination of leaf litter of *S. calophyllifolium* Walp. yielded in the isolation of 36 species belonging to 27 genera were recorded from the three grades of litter. An analysis of the composition of Mycoflora reveals that although a great majority of fungi isolated belong to Deuteromycotina, a few species belonging to Zygomycotina and Ascomycotina were also analyzed. Of the 36 species, 4 species in the 3 genera belonging to Zygomycotina, 2 species in 2 genera belonging to Ascomycotina. 30 species in 22 genera belonging to Deuteromycotina. Some species were found to be common to all the three grade of litters and a few are restricted to a particular grade of litter.

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