CHAPTER V
SUMMARY

The saprophytic fungi are versatile organisms and with their amazingly diverse species composition and ability to produce a variety of extra-cellular enzymes they act as regulators of the structure, function and dynamics of plant and animal community in nature. The nature and extent of mycoflora on plant litter, phylloplane, endophyte and soil is very complex and diverse and extensive literature is available on such studies carried out earlier. Amongst the microfungi isolated from varied substrates and habitats and so far recorded in literature, maximum species diversity was on decomposing plant litter.

This thesis embodies results of a study carried out during the last two years on the diversity, ecological association and activity of the sapophytic fungi associated with two locally grown plant species, *Ficus benghalensis* Linn. (F. Moraceae) and *Carissa congesta* Wight (F. Apocynaceae) and their immediate neighbourhood.

Partially decomposed leaf litter, young and mature fresh leaf, flower and fruit of *C.congesta* and *F. benghalensis* Linn., soil beneath the litter bed and air from 1 M above the ground level of the tree/bush canopy were the samples subjected for recovery of the associative fungi.

The leaf-litter and soil samples were subjected to particle plating (Bills & Polishook, 1994) and moist-chamber incubation (Hawksworth, 1974) techniques. Air samples were trapped in malt extract agar plates incorporated with antibiotics. Fresh leaves were subjected to a 3-step sterilization before the bits were planted in agar plates for the recovery of endophytic fungi. Conventional microscopic techniques and procedures were adapted for the study of fungi isolated.

A large number of isolates of fungi were recovered. All the sporulating fungi were identified down to the species level. Non-sporulating morphotypes were several and these were graded as 'dematiaceous' (coloured) and 'moniliaceous' (colourless) forms. Live cultures and herbarium specimens were maintained for all the fungi

described. Holotypes designated for the new taxa are housed in the herbarium of Goa University Fungus Culture Collection.

Sixty fungi that appeared commonly on leaf-litter, fresh leaf and soil samples and recovered in culture were subjected to amylase, cellulase, protease, pectinase, ligninase, laccase and xylanase enzyme assays using standard methods.

The data on fungal isolates were subjected to a '3- factor factorial completely randomised design analysis' defined in the 'Statistical Package for Social Sciences'. For endophytes, the '3^2 factor factorial completely randomised design analysis' of the SPSS was followed. To analyse the enzyme activity, 'Joining Tree Clustering' from "Statistica ver.5" was used. Square root transformations of the values obtained during the study was subjected to ANOVA Test. An exhaustive bibliography is given at the end of the thesis

About 5000 isolates of fungi were recovered. In all, these included 228 species of fungi belonging to 120 genera which included Mucorales, Hyphomycetes, Coelomycetes and Ascomycetes. Of these, 177 taxa belonging to 81 genera of Hyphomycetes, 4 species belonging to 2 genera of Mucorales, 12 species in 10 genera of Ascomycetes and 35 species belonging to 27 genera of Coelomycetes were recoverd. Sixteen hyphomycetes unlike any known species were not assigned to any known taxa since relevant literature was not available for identification. A total of 121 isolates did not sporulate in culture or on the substrate and recognised only as 'nonsporulating morphotypes' based on cultural characters. The detailed description, illustration and taxonomy of the mucoraceous and hyphomycetous fungi are given in the text. The Coelomycetes and Ascomycetes listed out here are not described in detail.

Of the 60 isolates subjected to enzyme assay, 29 showed positive activity against lignin and these were subjected for laccase and xylanase activity tests.

Twenty two species and 5 genera were new to science. These included: *Acronidiellina indica* sp. nov., *Cercospora carriseae* sp. nov., *Cirrenalia indica* sp. nov., *Dicyma carisseae* sp. nov., *Doratomyces indicus* sp. nov., *Gonatobotryum bimorphospora* sp. nov., *Hyaloscolecobasidium indicum* Gen. et sp. nov., *Idriella mucoidea* sp. nov., *Idriella multiseptata* sp. nov., *Kumbhamaya indica* Gen. et sp. nov., *Moorella ficusensis* sp. nov., *Neocercosporella indica* Gen. et sp. nov., *Nigrospora endophytica* sp. nov., *Paracylindrocladia indica* Genb. et sp. nov., *Parahumicola endophytica* Gen. et sp. nov., *Phialocephala carisseae* sp. nov., *Phialocephala nephrospora* sp. nov., *Phialomyces microsporus* sp. nov., *Sclerographium goanensis* sp. nov. and *Spadicoides indicus* sp. nov.

It is possible that particle-plating technique allowed the recovery of maximum diversity of fungi from the leaf particles. Eighteen taxa of well sporulating but unknown fungi have been recovered in this exercise.

In this investigation, many common (Hyphomycetes: *Beltrania, Beltraniella, Helminthosporium, Idriella, Phialocephala, Gyrothrix, Periconia, Scolecobasidium, Vanakripa, Volutella, Weisneriomyces* and *Zygosporium, Coelomycetes*: *Ajrekarella, Ascochytula, Botryodiplodia, Camarosporium, Coniochaet, Diplodia, Discosia, Lasidiploidea, Monochaetia, Neottiospora, Phyllostica, Robillarda, Seimatosporium, Stagonospora, Trichosprerma* and*Vasudevella*, Ascomycetes: *Diatrype, Diatrypella, Hypoxylon, Guignardia, Lophiostoma* and *Xylaria*, Mucorales: *Mucor)* and uncommon genera (*Aspergillus, Curvularia, Fusarium, Paecilomyces, Penicillium, Tritirachium, Torula, Botryodiplodia, Chaetomella, Chaetomium*, etc.) of fungi belonging to litter were recovered.

The species diversity studies revealed that there are a few abundant species and a high proportion of rare species of fungi associated with the two plant species.

The fungi present on all substrates, belonged to 17 genera of Hyphomycetes and 3 Coelomycetes. In all, 121 nonsporulating forms were recovered. The identity of these is not known.

The endophytic fungi isolated belonged to genera *Alternaria, Arxiella, Aspergillus, Beltrania, Cercospora, Curvularia, Cylindrocladium, Fusarium, Gliomastix, Hyaloscolecobasidium, Idriella, Nigrospora, Nodulisporium, Paecilomyces, Pseudobotrytis, Scolecobasidium, Stachybotrys, Wiesneriomyces,* a few Undetermined Hyphomycete taxa, *Ascochytula, Botryodiplodia, Discosia, Pestalotiopsis, Phomopsis, Robillarda, Septoria, Guignardia* and *Xylaria.* Some of these were also recovered from the litter.

The endophytic fungi often recovered from the upper layers of forest litter are associated with litter decomposition. The endophytes which survive as latent internal colonizers of fresh leaves are believed also to take an active part in the degradation of the litter. This kind of shift in function, from hidden dormant colonization to active degradation, may be considered as an ecological adaptation associated with litter decomposition. It is also possible that some of the litter degrading fungi might have adjusted and taken refuge as endophytes during some part in the year. The endophytes are certainly not a group of veiled refuges in the leaf tissues but an active defense system against herbivory by insects and higher animals.

The most dominant group of fungi in this study was the dematiaceous phialidic group (G2) and the least was moniliaceous blastic (G3). The coloured fungi in general (G2, G9 and G4) were the dominant groups. As in other plants, colour or pigments in fungi confers protection from stress of light and other harmful effects. It is therefore apparent that dematiaceous or pigmented fungi were well adapted as dominant litter colonizers.

Seasonal effect on the occurrence of fungi was highly significant. The recovery of fungi, especially the dematiaceous phialidic forms, was highest during

monsoon followed by the post monsoon months and the least in summer. The high humidity combined with high ambient temperature of the monsoon months in this part of the tropical region favoured growth and sporulation of a large number of fungi on plant substrates.

The number of fungi isolated from leaf litter of both plants was more than those on soil and in air. Along with the soil and air mycoflora, the endophytic fungi were represented in low magnitude. Although fungi visit other substrates and environment in the neighbourhood such as the soil and air, the eventual site of survival for dematiaceous fungi is the litter. It is therefore not surprising to come across a large number of fungi in the leaf litter.

The basal part of the leaf showed maximum diversity of ascomycetous and hyphomycetous fungi, during pre-monsoon and monsoon months, than in the middle and tip portion. In the structure and dynamics of leaf growth and function, basal portion is the most enduring part and it is natural to expect occurrence of maximum species diversity of fungi in this part. Endophytic fungi get into the leaf tissue at an early period of leaf growth and it is possible that during the course of its colonisation, the fungi consume considerable time and apparently are unable to reach the tip or edge of the leaf.

Of the 60 isolates subjected to enzyme assay, 29 showed positive activity against lignin and these were subjected for laccase and xylanase activity tests. The results showed that on the whole maximum percentage of isolates exhibited protease activity, followed by laccase, pectinase, ligninase, amylase, xylanase and the least were with cellulase activity. The total isolates associated with *Carissa congesta* showed highest percentage for cellulase activity, followed by amylase, ligninase, protease, pectinase, xylanase and laccase activity. The total positive isolates

associated with *Ficus benghalensis* showed high percentage for laccase, followed by ligninase, pectinase, amylase, xylanase, protease and the least of cellulase.

For laccase, promising results were shown by 9 Hyphomycetes, 4 coelomycetous and 5 nonsporulating forms. Some of the isolates are powerful producers of laccase. These included *Ajrekarella polychaetriae, Ascochyta caricae, Beltraniella buloloensis, Camarosporium indicum, Coniochaeta fuckelii, Cylindrocarpon ianthothele, Fusarium decemcellulare Gliomastix murorum, Periconia byssoides, Sporidesmium altum*, Undetermined hyphomycete sp. 13 and the nonsporulating isolates NS.34, 45, 47, 48 and NS. 121.

The fungal isolates showing the maximum number and highest and least laccase activity were isolated from *Carissa congesta.* Very few of the isolates from *Ficus benghalensis* exhibited laccase activity. Quantitative analysis for laccase of fungi associated with *Carissa congesta* showed that 78% of fungal isolates had low and 6% had high activity whereas with fungi associated with *Ficus benghalensis* showed 45% with low and none with high activity.

All these confirmed that fungi armoured with variety of enzymes undertake saprophytic mode of activities. Further, isolation of fungi alone will not give any indication of their activities. These fungi when tested for enzymes, showed that they are also very creative.

Building up an 'enzyme profile catalogue' along with collection of 'pure cultures' and setting up of a 'taxonomy database', as did in this work is an effort of conservation and utilization of fungal biodiversity. Further, study such as this will have a far-reaching implication in our efforts of building up of biotechnology resource supply bank in India.

Comprehensive floristic surveys on saprophytic and endophytic fungi are very few. Documentation and maintenance of pure cultures of fungi in state-of-the-art culture collections as done here are not attempted earlier. Screening of fungi for enzymes and other secondary metabolites were also not an organized effort so far in our country. Therefore, documentation, culture collection and screening as carried out in this work will be very useful and invaluable for future biotechnology and utilization in our country.

The strength of this work lies on the information documented on the biodiversity of fungi of India with special reference to this part of the country, better understanding on the ecological relation of occurrence of fungi on plants and their neighbourhood and the enzyme profile built up for several of those isolated fungi. All these are small but valuable additions to the knowledge and growth of fungal science in our country.