Materials & Methods
MATERIALS AND METHODS

*Mangifera indica* is cultivated abundantly around Tiruchirapalli, a city situated on the banks of the river Cauvery, in South India. The litter remains undisturbed in the mango groves. Hence the study has been undertaken to study the mycoflora of mango leaf litter. The litter has been collected from Tiruchirapalli and the neighbouring areas like Srirangam, an island surrounded by the rivers Cauvery and Coleroon and fertile lands.

For the present investigation, *Mangifera indica* leaf litter was selected. Litter samples were collected at bimonthly intervals from October 1998 to September 2000. The materials collected from the sites were brought to the laboratory in large sterile polythene bags. At the sample point, the litter was divided into 3 layers, namely upper layer (L) and fermentation layers (F₁ and F₂).

The upper layer (L) indicates leaves which have fallen recently, are light brown and undecomposed. They lie loosely on the surface and are dry. F₁ indicates leaves which are in the process of decomposition, but still recognizable, dark brown with a relatively high moisture content. They retain their structure and are compacted below the L leaves. Fungal activity in this layer is intense. F₂ indicates the leaves in advanced stages of
decomposition. They are fragmentary and are tightly compressed and are found below the F₁ leaves.

In addition to leaf litter, green mature leaves were also collected for the isolation of phylloplane mycoflora. These leaves were collected at random from different trees and cut with a pair of sterile scissors and brought to the laboratory in sterile polythene bags. The samples of living mature foliage were collected at bimonthly intervals on the same day as the litter collection.

Isolation of fungi from litter

Fungi were isolated from the litter samples by the following two methods.

1) Moist chamber incubation method

2) Dilution plate technique

Moist chamber incubation method

Twenty leaves were selected randomly from each set and were incubated in sterile moist chambers formed of large sterile Petri plates. The moist chambers were prepared by placing sterilized filter paper discs, which were moistened with sterile water. In order to prevent the samples from coming in direct contact with the sterile water, sterile glass bends were placed on the filter paper. High moisture content was maintained by moistening the
filter papers with sterile water periodically but without flooding the moist chambers. The leaves were incubated for a period of 48 hours and then examined under a binocular stereo dissection microscope for fungi. All fungi found in the sporulating stage were isolated, examined and identified.

**Dilution plate technique**

A sample of 5 leaves from each layer was randomly selected and from each leaf, five one cm. bits were cut with a pair of sterile scissors. Twenty-five bits thus obtained were placed in a 250 ml. Erlenmeyer flask along with 100 ml. sterile water. The flask was shaken for 30 minutes on a shaker in order to get a suspension of fungal spores. From this initial suspension several dilutions were prepared. 1 ml. of required dilution (1/1000) was pipetted out into each of the five replicate plates. Potato Dextrose Agar (PDA) medium was prepared for culturing the fungi. PDA with streptomycin sulphate 300 μg/ml. was cooled to 45°C and poured into each Petri plate. The plates were incubated at room temperature in a glass chamber for four days and then examined.

**Isolation of fungi from green foliage (phylloplane mycoflora)**

Ten green mature leaves were selected and from each leaf, five one cm. bits were cut using a sterile cork borer. The fifty bits thus obtained were placed in 250 ml. Erlenmeyer flask with 100 ml. of sterile water and shaken
for 30 minutes. One ml. of this suspension was pipetted out into 5 replicate plates. PDA with antibiotics was poured into the plates and were incubated at room temperature for four days and then examined for the phylloplane mycoflora.

**Slide preparation and photomicrography**

Plain lacto phenol and lacto phenol with cotton blue stains were used as mounting media for preparing semi permanent slides which were sealed with DPX mountant (supplied by BDH chemical division, Glaxo laboratories, India). Photomicrographs were taken with Nickon microscope using FX 35WA camera.

**Presentation of data**

In presenting the data, 2 terms, periodicity of occurrence and frequency of occurrence are used throughout the text. The term periodicity of occurrence is used to denote the number of samplings in which a particular fungus was recorded as against the total number of samplings. The total number of samplings was 12 both for litter and phylloplane. Based on periodicity of occurrence, the fungi were classified into 4 groups as follows.
The term frequency of occurrence is used to denote the number of leaves on which a particular fungus was present as against the total number of leaves examined (20) per layer by moist chamber incubation and is calculated as follows.

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\text{Frequency} (%) = \frac{\text{Number of leaves on which a fungus is found}}{\text{Total number of leaves examined (20)}} \times 100
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The frequency of occurrence of the fungi isolated by dilution plate technique is calculated as follows.

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\text{Frequency} (%) = \frac{\text{Total number of colonies of an individual fungal species in 5 replicate plates}}{\text{Total number of colonies of all fungi in 5 replicate plates}} \times 100
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