CHAPTER THREE
MATERIALS AND METHODS

The trees in the forests, orchards and fields in Nanded district (Maharashtra) were surveyed periodically from 1997 to 2002 to record the occurrence and distribution of various diseases of trees at different locations. There are more than 70 different tree species of horticultural, medicinal, commercial as well as of fuel wood importance, which were found under stresses during survey period.

Leaf, stem and fruit samples showing clear symptoms were collected in separate polythene bags from various sites. The infected different samples with distinct symptoms were brought to the laboratory for microscopic examination and for the cultural studies. All the specimens were carefully preserved to study the symptomology and etiology of diseases.

Natural as well as planted reserve forest of Kinwat range have not been explored for the tree diseases up till now. Therefore, this work has been undertaken with great curiosity.

Identification of fungi

The fungal pathogen can infect different plant parts, causing visible symptoms of the disease after the completion of the incubation period. The pathogens may be isolated from infected plant tissues such as leaves, stem, fruits and roots by applying different techniques for studying characters by which they can be identified. Fungal spore which is a characteristic feature
to identify a particular fungus may not be presented in the infected plant tissues. In all such cases, the infected parts of plants were placed in a moist chamber after surface sterilization by 0.1% mercuric chloride, maintaining high humidity. The spores or sporulating structures which were formed on the plant tissues were observed under microscope after 7 days incubation period. In laboratory, the freehand transverse sections of infected tissues were also taken immediately after the collection from field, and the causal agents were identified.

In order to study more morphological, cultural and pathogenicity details about the pathogen, isolations were made on potato-dextrose-agar medium.

The infected tissues were thoroughly washed with sterile distilled water. These infected tissues along with their small adjacent unaffected tissues were cut into small pieces and transferred by using flame sterilized forceps to sterile petridishes containing 0.1% HgCl₂ solution. The diseased tissue pieces were surface sterilized in this solution for 60 seconds and then washed in sterile distilled water for three times. The washed tissue pieces were aseptically transferred to petridishes containing potato-dextrose-agar nutrient medium supplemented with a pinch of streptomycin sulphate to avoid bacterial contamination. The plates were incubated at room temperature (25 ± 2°C). After incubation for 7 days, the fungal mycelium from petridishes were transferred on agar slants. The pure cultures of fungal pathogens were used for the precise identification. The
characteristics of the spores were studied and were used as a base of identification.

The fungal pathogens were identified by using various manuals, laboratory guides, reference books and available research papers.

For identification of fleshy fungi the macroscopic details, such as shape, size, colour of the basidiocarps were studied from fresh specimens (Bhatt et al., 1995).

**Identification of bacteria**

The diseased specimens were brought in the laboratory and the association of bacterial pathogen was confirmed by microscopic test. Isolations of bacteria were made on nutrient agar medium by following standard techniques and pure cultures of them were prepared. The pathogenicity of the isolates was confirmed by using Koch’s postulates (Deshpande and Papdiwal, 1978).

Other pathogens viz., viruses, mycoplasmas were identified on the basis of symptoms produced on the respective hosts, and their comparison with the descriptions mentioned in the reference literature.

The symptoms of many of the diseases were recorded in the field, specially of abiotic factors, termites, insects and decay of trees or wood.

For studying post harvest diseases of fruits, the samples were collected from the fields as well as from local market. Their symptoms were recorded and the pathogens were identified in the laboratory by the usual methods.