Plants play an important role in the functioning of the living world and are an integral part of the ecosystem. They are also the primary source of medicine. Vedic literature and findings in ancient civilization have shown that plant based medicines find application to cure human and animal diseases. It is estimated that approximately 80% of the world's population still use herbal medicine and supplementation for their primary health care. Forests are an important source of herbal medicine and are being subjected to anthropogenic pressure. This has resulted in the depletion of medicinal plants in the natural habitat. In addition to this, forest fire, pests and diseases also bring about a significant decrease in plant population.

Cultivated food crops have been thoroughly studied for their diseases and pests, which has resulted in the development of new crop varieties and pathogen strains. Due to an increase in the demand for medicinal plants, certain medicinal plants of international commerce are being cultivated in large scale in agricultural lands. Just like agriculture crops, pathogenic microorganisms also infect even the medicinal plants. On the other hand, uncultivated medicinal plants have not been studied for their diseases.

A survey of literature revealed that a few reports are available on diseases of herbaceous plants often found growing in the wild or in uncultivated area (Nath and Bhargava, 1980; Tripathi, 1985; Aneja and Kaushal, 2000 and Singh and Bhalla, 2000). Some of the reports also highlight the effect of pathogenesis on the ability of the medicinal plants to produce primary and secondary metabolites (Sharma et al., 1993;
Nagaraja, 1988; Sindhan and Parashar, 1996 and Bohra and Purohit, 2003). The contents of some of the important secondary metabolites like essential oils, alkaloids, flavonoids and polyphenol content have been shown to alter following fungal infection (Prasad and Sah, 1991; D'Aulerio et al., 1995, Hosagoudar et al., 1997 and Chakraborty et al., 2002).

Bhadra Wildlife Sanctuary (BWLS) is an abode for medicinal plants and is situated in the heart of the Western Ghats. A variety of medicinal plants grow naturally in this place and a good many number of them are endemic. There has been no information on the diseases of medicinal plants growing naturally in the sanctuary. Hence, the present study was taken up.

The objectives of the present investigation are, to survey of herbaceous medicinal plants in the sanctuary for their diseases, severity, seasonal incidence and damage caused to them in terms of change in primary and secondary metabolites qualitatively and quantitatively and to isolate and characterize the fungal pathogens associated with diseased plants and to test them for their pathogenesity in their respective hosts.

Thirty-six study sites were selected which are located in 12 state forest regions within BWLS area. In each of the study sites, there were three replicates, each consisting of 3 quadrates (10x10 sq m). Surveys of these study sites were conducted for herbaceous medicinal plants during the 1st year and their identity was established based on standard identification manuals (Saldana, 1976; Gamble, 1997). Subsequent visits were made at least once in the season during Jan 2001 to Dec 2002. During this period, diseased medicinal plants were identified, collected, brought to the laboratory and studied for disease symptomatology and the associated fungi. Fungi associated with these diseased samples were characterized by incubating them on wet blotters and PDA contained in
petri dishes. The characterization of fungi was based on their colony habit, fruiting bodies, spore characteristics and pigmentations, with the help of identification manuals (Barnett, 1972 and Subramaniyam, 1983). The dominant fungus associated with these infected materials was determined by counting their presence in segments of incubated infected materials. Further, pathogenesis test was conducted by leaf/stem bioassay; where in, the spores of fungi on PDA culture were inoculated artificially to fully expanded leaves or stem. In case of the fungus failing to sporulate on culture, they were cultured individually on autoclaved toothpicks, axenically, which were then used for artificial inoculation of stem. The disease symptoms expressed on leaves/stems were compared with the symptoms of infection in naturally infected plants. The disease symptomatology was studied. The disease symptoms obtained by artificial inoculation were incubated on wet blotter or PDA. The disease incidence of species in each of the study area was determined based on the number of infected plants out of the total number of plants in an area. Disease incidence was determined only on selected 13 medicinal plant species like, *Centella asiatica, Clitorea ternatea, Cyclea peltata, Dioscorea alata, D. bulbifera, Elephantopus scaber, Hemidesmus indicus, Ichnocarpus frutescens, Naravelia zeylanica, Rauwolfia serpentina, Rubia cordifolia, Tinospora cordifolia and Withania somnifera*.

In each season, the study sites were visited at least once and the data on disease incidence was collected. Based on the data of fungal diseases of medicinal plants in each of the study site, in the whole of the sanctuary, a disease map was prepared for each of the medicinal plant.
The disease materials of select plants were collected from study sites depending on the season and were graded into apparently healthy, partially infected (the available moderately infected samples) and the totally infected leaves. The infected and uninfected samples were air-dried under ambient conditions of the laboratory and stored in airtight zipper-lock polyethylene covers and stored in a dark cool place. These samples were tested for primary metabolites like total carbohydrates, proteins, prolines and lipids (Hedge and Hofreiter, 1962; Lowry, 1951 and Anon., 1957) and secondary metabolites like alkaloids, flavonoids, phenolics and sterols (Ikan, 1969; Swain and Hillis, 1959; Folin and Denis, 1939 and Sanchez et al., 1972).

First, the samples were subjected to preliminary screening by qualitative analysis of the above secondary metabolites (Fiegel, 1960; Gibbs, 1974) and then they were subjected to quantitative analysis following their positive response to qualitative tests.

Results of the present investigation indicated that there are 158 species of plants of 122 genera and 50 families. The distribution of these species depended on geographical location and climatic condition. Two distinct vegetation types have been noticed - moist deciduous and dry deciduous forests. The state forest region Muthodi has large number of herbal species followed by Kagemanegiri and Aldara and the rest of the state forest regions had low species incidence.

Medicinal plants of different study sites expressed foliar disease symptoms in the form of leaf spot or leaf blight and rarely, the other above ground parts were found infected. The below ground plant parts could not be tested for their diseases and disorders since, their extraction is prohibited in the sanctuary. Out of 158 species, nearly 50% of them were found to affected by foliar diseases due to fungal pathogens in one or the other
seasons or study sites. The incubated fragmented foliar disease materials produced more than one fungal species, which included common saprophytes as well as field fungi. However, certain species of fungi were found aggressively expressing themselves in the form of sporulation or fruiting body and these were considered as major associates. Only one fungus and rarely two, when artificially inoculated to leaves and stems produced symptoms of disease very similar to that produced in naturally infected plants. However, certain other fungal species also caused symptoms, which were dissimilar. In most cases, it was those fungal species, which occurred in majority on infected leaf samples that caused symptoms of disease. Some of the pathogens that produced symptoms of disease are Alternaria alternata, Cercospora centellae, Colletotrichum dematium, C. gloeosporioides, C. lindamuthianum, Pestalotioptis macrotrichia and Curvularia lunata.

The occurrence of disease in the selected medicinal plants depended on the season, and prevalence of optimum temperature and relative humidity, vegetation type and also the geographical location. The disease incidence in most selected medicinal plants was high during the rainy season, which gradually decreased during winter and summer seasons. Certain diseases caused by Alternaria and Cercospora were more prevalent during post-rainy season. Some of the diseases, which increased during the rainy season, are Colletotrichum and Pestalotiopsis leaf spot disease on Dioscorea, Hemidesmus, and Ichnocarpus and on the other hand, Alternaria and Cercospora foliar disease in species of Cyclea, Rauwolfia and Centella were common during post rainy season. The disease maps prepared for each of the selected medicinal plants growing in the sanctuary area indicated that most foliar diseases are commonly prevalent in the
entire sanctuary area. Only a few plant species, which could be identified in specific study sites, have diseases limited to certain areas. The preparation of disease map for each of the selected medicinal plant helps to monitor the disease incidence in a particular season and disease spread over a period of time.

Quantitative estimation of the primary metabolites like carbohydrates, proteins and lipids indicated that fungal infection in all medicinal plants caused decrease in their contents. The extent of reduction increased with increase in infection, that is, in 100% infected materials, the decrease was very drastic while, in moderately infected leaves, it was less than in the uninfected leaves but, it was considerable.

The qualitative estimation of secondary metabolites suggested that the presence of alkaloids, steroids, polyphenolics, glycosides, triterpenoids and flavonoids in most of the select medicinal plants. This further indicated that the secondary metabolites could be estimated quantitatively by standard analytical techniques. The quantitative estimation of alkaloids and steroids indicated that the fungal disease in these medicinal plants could cause significant decrease in the content; on the contrary, certain other secondary metabolites like flavonoids and polyphenols increased in diseased plant materials. The decrease in the content of primary metabolite as well as some of the secondary metabolites could be attributed to the process of pathogenesis by fungi that are known to alter the metabolic process(es) of the host plant. The reduction in primary metabolite content could be attributed to their metabolism by fungi during their growth and establishment. Secondary metabolites, flavonoids and polyphenols increased in infected plant material since they are produced in large quantities in host plants in response to fungal infection. These two secondary metabolites have been shown to produce in
defense in most plant species (D'Aulerio et al., 1995; Sindhan and Parashara 1996 and Hosagoudar et al., 1997).

The investigations of present study indicated that medicinal plants growing naturally in the sanctuary area are affected by fungal diseases, whose incidence varied depending on the host plant, environmental condition and geographical location. Although, a large number of fungal species are associated with disease symptoms only one or two dominated the incubated leaves. When artificially inoculated it is these dominant fungi that caused symptoms of disease very much similar to that produced in nature. The disease caused by these fungi varied depending on the season and the host. The disease map for each of the selected medicinal plants, established the fact that the foliar diseases due to specific pathogens are distributed widely in the sanctuary area. Disease maps also gave an indication of disease incidence in different seasons and over a period of time. The quantitative analysis of primary metabolites like carbohydrates, proteins and lipids, as well as certain secondary metabolites like alkaloids and steroids indicated that their content decreased due to fungal infestation. However, secondary metabolites like polyphenols and flavonoids increased, following infection, suggesting that the infected plants are on defense. Further, work on the qualitative changes in secondary metabolites of fungal infested plants is required for the proper understanding of changes in medicinal principles following fungal infection.