Introduction

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say ‘Return to Nature’.

Plants have long served as our major source of medicinal compounds. The earliest writings from ancient Babylonia, Egypt, China and India were found with references to healing herbs, indicating a prehistoric origin for the use of plants as medicines (Sumner, 2000). *Ayurvedic*, as the name implies (*Ayu*: Life, *Veda*: Knowledge) is the knowledge of healthy living and is not confined only to treatment of illness (Dahanukar *et al*., 1989). Drug therapy based on traditional system of medicines employing herbs forms a major therapy because of its no side-effect. Therefore, medicinal plants play an important role in the development of potent therapeutic agents.

Indian subcontinent is endowed with varied agroclimates and topography. Therefore, it has the richest plant biodiversity of the world and is rightly acclaimed as the ‘Botanical garden of the world’.

1.1 Ayurveda - the ancient science of life

Among the ancient civilizations, India has been known to be the rich repository of medicinal plants. The *Rig-Veda* (5000 B.C.), *Yajurveda* and *Atharveda* (4000-2500 B.C.) mentioned 67, 81 and 290 species of medicinal plants, respectively. *Charak Samhita* (700 B.C.) and *Sushruta Samhita* (200 B.C.) have described properties and uses of 1,100 and 1,270 plants, respectively in compounding of drugs and these are still used in classical formulations in the Ayurvedic system of medicines. Unani and Siddha system of medicines also utilize a number of plants for medicinal purpose. India accounts for 8% of global biodiversity which exists in 2.4% land area of the world. There are 15,000 species of higher plants, of which about 10% are found to have medicinal properties out of these only 800 plants species are used in traditional system of medicines in India (Joy *et al*., 2001 and Sinha *et al*., 2002).
Herbal drug constitutes only those traditional medicines that primarily use medicinal plant preparations for therapy. The ancient record evidencing their use by Indian, Chinese, Egyptian, Greek, Roman and Syrian people dates back to about 5000 years. About 500 plants with medicinal use are mentioned in ancient texts and around 800 plants have been used in indigenous systems of medicine. The traditional medicine is increasingly solicited through the tradipractitioners and herbalists in the treatment of infectious diseases. Among the remedies used, plant drugs constitute an important part (Samy and Gopalakrishnakone, 2007).

Traditional medicines are used by about 60 per cent of the world’s population. These are not only used for primary health care in rural areas in developing countries, but also in developed countries as well where modern medicines are predominately used (Kamboj, 2000). In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reportedly using herbs to treat medical illness during 2003 (Bent, 2004). Meena et al. (2009) stated that herbal medicines have been main source of primary healthcare in all over the world.

Herbal medicines are finished, labelled medicinal products that contain active ingredients, aerial or underground parts of plant or other plant materials, or their combination, whether in the crude state or as plant preparations.

Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 (Seth et al., 2004).

Present scenario states that more than 30,000 plant species of medicinal importance are grown all over the world. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries (Raghavendra et al., 2009).

1.2 The importance of medicinal plant

A medicinal plant is any plant which is used in order to relieve, prevent or cure a disease or to alter physiological and pathological process, or any plant employed as a source of drugs or their precursors.
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Therefore, medicinal plants play an important role in the development of potent therapeutic agents. The use of various herbal remedies and preparations are described throughout the human history representing the origin of modern medicines. Plant based drugs provide outstanding contribution to modern therapeutics. They contain a variety of different nutritious and therapeutic constituents: vitamins, minerals, trace elements - as well as active ingredients with a variety of medicinal actions. These include volatile oils, tannins, mucilage, alkaloids, bitters and flavonoids. For example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure (Verma *et al.*, 2008).

There has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. So, the large scale use of medicinal plants and herbs in the preparation of drugs is increasing both in developed and developing countries due to growing concern about the side-effects of chemicals and synthetic substances used in medicines which are considered to be responsible for causing ill effects (Fransworth *et al.*, 1985; Fransworth, 1987; Dhawan and Rastogi, 1991; Xiao, 1991; Cox and Balik, 1994). Plants are also found to contain disease specific curative properties and extracts of such plants are increasingly being used to manufacture effective therapeutic and preventive drugs. Because of these factors the demand for plant-based medicines is increasing worldwide, as it is reported that synthetic drugs have some undesirable consequences for human health (Verma and Dubey, 2006).

Medicinal plants are valued for the content and chemical composition of their active components, thus constitute one of the few natural resources, which are being overexploited for the benefit of the mankind. Like other natural resources, they are also limited and face the danger of extinction due to over exploitation. An attempt to conserve and protect these plants presents a formidable challenge. Keeping this in view, conservation of these plants becomes very important not only for conservation of the genetic diversity *in situ* but also to put to judicious and efficient utilization on a sustainable basis. India is one of the major exporters of medicinal plants and this has led to cultivation of medicinal plants on a large scale. Commercial cultivation has also increased the disease problems in medicinal plants.
1.3 Development of disease

Plant disease can be defined as an interaction between a host, a pathogen and the environment that results in harmful alternations in the physiology and structure of the plant. Disease development is a dynamic process. It depends on:

- The dissemination of the pathogen to the susceptible host.
- The environmental conditions prevailing at that time.
- The reactions of the host

Medicinal plants are also attacked by fungi, bacteria, phytoplasmas, viruses, nematodes and mycoplasmas causing diseases and some of these are notably notorious and lead to epidemics in favourable climatic conditions. Not much attention towards the cause and control of these diseases has been paid so far (Janardhanan, 2002).

1.3.1 Diseases of Medicinal Plants

Plants continue to be the major source of drugs, in spite of the phenomenal development in the synthetic compounds during the last two decades. Due to the realization of the toxicity and side effects associated with the synthetic drugs, the importance of the plant-based drugs has enhanced substantially in the recent years. This resulted in the increased demand of medicinal plants manifold. Since the availability of a number of important medicinal plants from natural sources was insufficient to meet the demands of pharmaceutical industries, large-scale cultivation of these plants has come into practice as monocultures. Commercial cultivation dramatically increased the disease problems, number of diseases and their severity in these plants. Like any other plants, medicinal plants also get devastating attacks of injurious diseases when they are being exploited commercially. Sometimes, their conservation becomes extremely important fearing their extinction due to over exploitation and in several other occasions the diseases affecting these medicinal plants restrict their cultivation and production.

Wind is particularly considered as an effective means of disseminating foliar pathogens. The mechanism of spore release by wind is that increasing wind speed decreases the thickness of the boundary layer of air at the leaf surface, thus exposing more deeply immersed spores to the action of eddies (Meredith, 1973). Examples of this in the field are seen in spore trapping results on *Alternaria* species. Thus, both
Rotem (1964) working on *A.solani*, and Meredith (1966) working on *A.porri* have recorded successive increases in atmospheric content of these fungi with successive increases in wind velocity.

Rain splash is well recognized as an agent in the release and dispersal of spores of many fungi. For example water may also bring about release of certain “dry-spored” fungi such as *Botrytis* spp. Conidia of *Colletotrichum coffeanum*, which causes coffee berry disease (CBD) are released and dispersed in the absence, of vectors solely by water-splash over comparatively short distances. Thus, splashing rain and irrigation water are frequently of prime importance in the spread of fungal pathogens. Pathogens cause more damage to the plant by secreting toxins, enzymes, and other chemicals that impair the plant’s physiological processes. In general, fungi cause local or general necrosis of plant tissues, and they often cause reduced growth (stunting) of plant organs or entire plants. Symptoms of disease include death and destruction of host tissue, wilting, abnormal growth and differentiation and discolouration of host tissue. The necrotic lesions caused by pathogens can be localised or extensive. Discolouration of tissue is most commonly by chlorosis or mosaics of leaves, both of which can have a number of causes. Anything that interferes with the production of chlorophyll causes leaves to turn yellow, or chlorotic.

**Table 1.3.1.1** Some of the most common terms used to describe symptoms of plant diseases.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Diseases</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blight</td>
<td>A disease characterised by widespread death of plant tissue.</td>
</tr>
<tr>
<td>2.</td>
<td>Downy Mildew</td>
<td>White or grey 'bloom' on leaves and stems caused by production of sporangiophores and sporangia by members of <em>Peronosporales</em>.</td>
</tr>
<tr>
<td>3.</td>
<td>Mosaic</td>
<td>Patchy variation of normal green colour in leaves, usually light and dark green mosaic, symptomatic of many viral diseases.</td>
</tr>
<tr>
<td>4.</td>
<td>Powdery mildew</td>
<td>White powdery ‘bloom’ on the plant surface caused by the production of fungal mycelium, conidiophores and conidia by members of the <em>Erysiphales</em> (powdery mildew fungi).</td>
</tr>
<tr>
<td>5.</td>
<td>Rust</td>
<td>Rust-coloured pustules formed by members of the <em>Uredinales</em> (rust fungi).</td>
</tr>
</tbody>
</table>
The most common necrotic symptoms are leaf spots, blights, canker, die-back, root rot, damping-off, basal stem rot, anthracnose, scab and wilt. Fungal diseases namely foliar, stem and root diseases play an important role in affecting overall health of the medicinal plants.

Certain abiotic factors are also responsible for causing foliar diseases in medicinal plants like deficiencies in soil, lack of proper irrigation, moisture stress, temperature extremes, unfavourable light conditions, naturally occurring toxic chemicals, pollutants, etc.

Table 1.3.1.2. Major foliar diseases of Medicinal Plants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fungal Diseases</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Alternaria</em> leaf spot</td>
<td>Small, chlorotic, water soaked lesions appear on both surfaces of the leaflets. These lesions enlarge, become irregular in shape and are brown with darker brown margins; the central portions later become pale, dry rapidly, and disintegrate. Sometimes the spots show concentric rings.</td>
</tr>
<tr>
<td>2.</td>
<td><em>Alternaria</em> leaf blight</td>
<td>Light to dark brown irregular spots appear towards the tips or margins of the leaves. These rapidly enlarge and join together to form wedge shaped lesions. In the later stages of disease development, blighted areas become dark brown and tend to break.</td>
</tr>
<tr>
<td>3.</td>
<td>Scab</td>
<td>Numerous round to irregular lesions appear on both surfaces of leaflets. The lesions on upper leaflet surfaces are light tan with sunken centers and raised margins. Lesions are frequently covered with continuous velvet-like layers of grayish olive green fruiting bodies.</td>
</tr>
<tr>
<td>4.</td>
<td><em>Cercospora</em> leaf spot</td>
<td>Small necrotic lesions appear on leaflets. These lesions enlarge and become irregular or angular shaped light brown spots. When long periods of leaf wetness occur, the spots join together leading to drying of leaves and defoliation.</td>
</tr>
<tr>
<td></td>
<td>Disease Name</td>
<td>Description</td>
</tr>
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<tr>
<td>5.</td>
<td>Powdery Mildew</td>
<td>Large whitish spots cover the upper surfaces of leaflets. These spots are covered with fungal growth that gives them a powdery white appearance. The centers of the spots later become brown and necrotic. Powdery mildew sporulates on upper and sometimes on lower leaf surfaces.</td>
</tr>
<tr>
<td>7.</td>
<td>Phyllosticta leaf spot</td>
<td>Infection starts in damaged and dead tissue and subsequently spreads into the living green areas of the leaflets. Lesions are circular to irregular and are light tan in color surrounded by a reddish-brown border.</td>
</tr>
<tr>
<td>8.</td>
<td>Myrothecium leaf blight</td>
<td>Round to irregular lesions with gray brown colored centers and brown margins surrounded by chlorotic halos occur on both surfaces of leaflets. When lesions join together, give dried appearance on leaves.</td>
</tr>
<tr>
<td>9.</td>
<td>Sclerotium leaf spot</td>
<td>Appear on mature plants as gray necrotic ring spots which may develop shot holes. During long periods of leaf wetness the spots coalesce leading to a severe blight.</td>
</tr>
<tr>
<td>10.</td>
<td>Anthracnose</td>
<td>Wedge-shaped lesions appear on the leaf tips. Lesions may also develop on the leaf margins leading to marginal blight. The periphery of the advancing margins of the lesion is surrounded by a bright yellow zone.</td>
</tr>
<tr>
<td>11.</td>
<td>Pestalotiopsis leaf blight</td>
<td>Dark brown circular spots surrounded by faint yellow haloes appear on infected leaves. These spots enlarge and join together to dry up the leaflets, especially on the margins.</td>
</tr>
</tbody>
</table>
1.4 Phyllosphere

The Dutch microbiologist Ruinen coined the term Phyllosphere which is the interrelationship between plant foliage and the quality and quantity of microorganisms found on the surface. The leaf surface is termed as phylloplane. The phyllosphere represents a niche with great environmental significance. There is growing evidence for important interactions of phyllosphere microbial inhabitants which may affect the fitness of natural plant populations and the quality and productivity of certain vital medicinal plants and agricultural crops.

Phylloplane provides a suitable habitat for the growth of antagonistic microorganisms which can compete with the pathogen for nutrients and inhibit pathogen multiplication by secreting antibiotics or toxins. The quality and quantity of the microorganisms on the leaf surface differs with the age of plant, leaf area, morphology and atmospheric factors (temperature, humidity etc.). Therefore, aerial plant surface is considered as a dynamic environment (Fokkema, 1976).

The position of the leaf also plays a role in determining the microflora. Moreover, plant leaves are exposed to dust and air currents resulting in the establishment of the typical microflora on their surfaces aided by cuticle, waxes and appendages (thorns, spikes) which help in the anchorage of the microbes. The principle nutritive factors in the leaf are amino acids, glucose, fructose and sucrose.

1.4.1 Leaf surface as a microbial habitat

The leaf provides limited nutrient resources to the residing microflora on its surface. Leaf itself is surrounded by a very thin laminar layer in which moisture emitted through stomata may be sequestered, thereby alleviating the water stress to which epiphytes are exposed.

Some cells in a leaf affects bacterial or fungal population, particularly in plant-pathogenic populations, which may not reside in exposed sites on the leaf surface but instead they may at least locally invade the interior of the leaf, avoiding the stresses on the exterior of the leaf by residing in substomatal chambers or other interior locations (Mercier and Lindow, 2000; Wilson et al., 1999). Thus, while some phytopathogens may have the option of avoiding stresses, but most of the other
epiphytes apparently tolerate them in some way or the other (Beattie and Lindow, 1995, 1999).

Cuticle the waxy layer possesses three-dimensional crystalline structures on different plant species and can change as leaves age, presumably in part due to microbial modifications (Knoll and Schreiber, 1988, 2000 and Mechaber et al., 1996) limit passive diffusion of nutrients and water vapour from the plant interior on to the surface and defines the hydrophobicity of the leaf.

1.4.2 Microbiology of Phyllosphere

Fungi that live on the aerial parts of plants have been defined as endophytes if they live inside the plant tissues and as epiphytes if they live on the surface of their host (De Barry, 1866; Arnold, et al., 2000; Inacio et al., 2002; Lindow and Brandl, 2003).

The surfaces of aerial plant parts provide a habitat for epiphytic microorganisms, many of which also influence the growth of pathogens. Bacteria are generally the predominant initial inhabitants of newly expanded leaves, while yeasts and filamentous fungi dominate later in the growing season (Kinkel et al., 1987). The phyllosphere is the three-dimensional space on the leaf surface. The environment of the phyllosphere includes physical, chemical and the biological components occupying the surrounding space. In recent years, considerable attention has been paid to the components of the microflora present on the leaf surface, a specialized habitat commonly known as the phylloplane.

The nutrient status of water films is usually low but may be increased, especially as leaves grow older, by leakage from their cells and deposition of materials such as honey dew from aphids and pollen. The resident microflora and leaf pathogens compete for nutrients, and the results of such competition and antagonistic reactions between them may largely determine disease severity.

Some pathogens are partly controlled by microorganisms that occur naturally on aerial surfaces of plants and many attempts have been made to improve control by applying selected antagonists to such surfaces. Antagonists often compete for nutrients with the pathogen, and antibiotics may be formed that reduce germination of its spores and subsequent growth. Hyphae of fungal pathogens may be killed on contact with the antagonist or by direct penetration. The plant’s defence may be
stimulated before challenge by a pathogen. Apart from killing the pathogen, an 
antagonist may reduce its reproductive capacity.

1.4.3 Ecology of Phyllosphere

The microflora present on the leaf surface, which is a specialized habitat 
commonly known as the phylloplane. Environmental factors severely restrict the 
growth of microorganisms on leaf surfaces. The microbial communities of 
phyllosphere are diverse, supporting numerous genera of bacteria, filamentous fungi, 
yeasts, algae, and less frequently protozoa and nematodes which may form resident 
populations on leaves (Morris et al., 2002; Lindow and Brandl 2003; Andrews and 
Harris 2000; Beattie and Lindow 1995; Kinkel 1997; Lindow and Leveau 2002). The 
non-pathogenic fungi that inhabit the phyllosphere depend on nutrients exuded from 
the leaf or those deposited from the atmosphere (Belanger and Avis, 2002; Inacio et 
al., 2002). Microbes reach the phyllosphere by atmospheric deposition from plant and 
soil sources, but may also colonise plants through the roots, and become transported 
to aerial parts.

Of the bacteria most are Gram-negative, often chromogenic, and include the 
genera: Erwinia, Pseudomonas, Xanthomonas, and Flavobacterium. Gram-positive 
bacteria such as Lactobacillus, Bacillus, and Corynebacterium are isolated less 
frequently. In addition to saprophytic bacteria, pathogenic bacteria, e.g. Pseudomonas 
syringae pv. syringae, P.syringae pv. morsprunorum, P.syringae pv. glycinea, 
Erwinia amylovora, and E.carotovora, can live in a nonpathogenic epiphytic phase on 
foliar surfaces (Biswas et al., 2007).

Ecological competence for phyllosphere colonization involves development of 
both pathogen and antagonist in the phyllosphere which is determined by several 
abiotic factors such as availability of nutrients, temperature, water availability, UV 
radiation and the deposition of agrochemicals (Breeze and Dix, 1981; Sundin, 2002; 
Zak, 2002). Water is frequently limiting on plant surfaces under temperate and arid 
conditions, which eventually affects the growth of epiphytic microbes which may 
only occur during rain, periods of dew, or at least high humidity (Campbell, 1989). 
Colonization ecology of phylloplane and/or phyllosphere fungi principally relates to 
the prevailing microenvironmental conditions on the leaf surfaces and their physical,
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Biological control of some important foliar diseases of Medicinal Plants

Chemical and phenological properties which affect the fungal establishment (Pandey 1990; Dix and Webster 1995).

Water affects survival, multiplication, and dispersal of plant surface bacteria, and free water is probably necessary for diffusion of antimicrobial compounds from epiphytic bacteria used as biocontrol agents. On healthy plant surfaces, important nutrient sources are mainly deposits. Pollen grains deposited on the leaf surface leaching high amounts of easily degradable nutrients such as amino acids and sugars, are an important nutrient source in the phyllosphere (Andrew, 1990).

Thus, the phyllosphere is regarded as a complex system. To understand it will require knowledge of:

- Atmospheric components (Aerobiology, Meterology).
- Physiology and anatomy of the host plant.
- Relevant diseases, including foliar pathogens, root pathogens and air pollution.

Atmospheric components:

The atmospheric microflora can vary in composition and concentration diurnally and seasonally as well as in response to environmental events such as rainfall and high wind (Kinkel 1997; Zak 2002), directly influencing the immigration of microorganisms to the phyllosphere. Agricultural practices such as harvesting and cultivation also influence atmospheric microbiology and colonisation of nearby plants (Lindemann et al. 1982; Lacey 1996; Lighthart 1997). Immigration of microorganisms to leaves from the atmosphere can take place through impaction onto the leaf surface, sedimentation or rain splash as well as from contamination with soil (Venette and Kennedy 1975; Lacey 1996). Certain meteorological factors such as temperature, relative humidity are found to be responsible for change in microbial population.

Physiology and anatomy of leaf surface of host plant:

Anatomical and physiological characters of the leaf surface and its physico-chemical environment substantially influence the density and diversity of phyllosphere - inhabiting microorganisms, which may include natural antagonists of important pathogens. Plant species, leaf age, leaf position, physical environmental condition and availability of immigrant inoculum have also been suggested to be
involved in determining species of microbes in the phyllosphere (Andrews et al. 1980; O’Brien and Lindow, 1989; Wilson and Lindow, 1994). Leaf surface topography and nutrients present on the leaf surface are generally recognized as important regulators of phyllosphere microbial communities, while little research has been done at the whole community level (Hirano and Upper, 2000; Yadav et al. 2005; Yadav et al. 2008).

The nature and abundance of epiphytic and endophytic leaf fungi have been studied mainly in forests, but their investigation with reference to host parasite are less explored (Heredia, 1993; Hata et al. 2002). Results showed that microflora are found to exhibit high degree of host specificity.

De Costa et al. (2006) studied leaf anatomical and physiological characters related to variation of phyllosphere microflora of different rice varieties in Sri Lanka. They characterize leaf area, leaf hair density, leaf hair length, stomatal density on upper and lower leaf surfaces, leaf thickness and specific leaf fresh weight (i.e. leaf fresh weight per unit leaf area) as anatomical characters of rice leaves. While, the physiological characters measured were stomatal conductance, transpiration rate and leaf temperature. During experimental study they analysed the significant variation in the density and diversity of phyllosphere epiphytic microbial population among the rice varieties tested. They also studied that microbial populations under field conditions can fluctuate substantially because of the direct exposure of the phyllosphere to extreme environmental conditions such as high solar radiation, UV radiation, wind and rain (Romantschuk, 1992).

**Effect of air pollutants and particulate matter on leaves:**

An atmospheric particulate matter is a mixture of diverse elements and are found to be responsible for causing the plant diseases. Effects of particulate matter on vegetation may be associated with the reduction in light required for photosynthesis and an increase in leaf temperature due to changed surface optical properties (Prajapati, 2012). Leaves are the most susceptible and highly exposed parts of a plant, which may act as persistent absorbers in a polluted environment (Maiti, 1993).

Leaf orientation, age, roughness and wetability of the leaf surface influences dust interception and thus retention (Neinhuis and Barthlott, 1998; Beckett et al., 2000). The strength and constancy of wind, the porosity of the vegetation with respect
to air movement also affect dust retention (Raupach et al., 2001). Exposure to a given mass concentration of airborne particulate matter may lead to widely differing phytotoxic responses, depending on the particular mixture of deposited particles. Particulate deposition and effects on vegetation unavoidably include (1) nitrate and sulfate and their associations in the form of acidic and acidifying deposition and (2) trace elements and heavy metals, including lead. While size is related to mode and magnitude of deposition, and may be a useful substitute for chemical composition (Whitby, 1978).

Dusts with pH values of $\geq 9$, may cause direct injury to leaf tissues on which they are deposited (Vardak et al., 1995) or indirectly through alteration of soil pH (Auerbach et al., 1997) and dusts that carry toxic soluble salts will also have adverse effects on plants (Prajapati and Tripathi, 2008 a-d). Dust accumulating on leaf surfaces may interfere with gas diffusion between the leaf and air. Sedimentation of coarse particles affects the upper surfaces of leaves more (Thompson et al., 1984; Kim et al., 2000) while finer particles affects lower surfaces (Ricks and Williams 1974; Fowler et al., 1989; Beckett et al., 2000).

1.5 Disease Management

All plant diseases result from a three-way interaction between the host, the pathogen and the environment. An epidemic develops if all these factors are favourable to disease development. Therefore, disease can be controlled by manipulating one or more of these factors so that conditions are unsuitable for replication, survival or infection by the pathogen. Disease surveys are important and are really the first step in the application of control measures. Control measures must be economically feasible - generally expenditures must be commensurate with the expected benefits.

1.5.1 Chemical Control

Chemical disease control employs the use of chemicals that are either generally toxic and used as disinfectants or fumigants or chemicals that target specific kinds of pathogens. Ideally, a chemical control agent should be effective at concentrations that will not harm the plant, having low risk to humans and animals, and causing minimal effect on the normal microflora on the plants and in the soil.
Considering the great socioeconomic and therapeutic importance of medicinal plants it is imperative to control these diseases. The use of chemicals has lead to dramatic improvements in the production of crop plants. Since such improvements provided a reliable supply of cheap food they were initially welcomed. Of late, consumers are becoming increasingly concerned both about food quality and of the real and imagined effects of modern farming methods on the natural environment. Problem associated with the use of fungicides such as environmental pollution, deleterious effect of chemicals on non-target organisms, fungicidal resistance in pathogens and resurgence of target pathogens, outbreak of secondary pathogens and escalating costs of developing and applying chemicals are known to affect the vitality of plants and the well being of the society (Naik and Sen 1992).

The excessive use of fungicides and other agrochemicals, imparts a hazardous impact detrimental to human and other living organisms. Control with chemicals is uneconomical and not advisable to avoid ground water pollution, death of non-target beneficial flora and fauna and risk of new resistant pathogen strains. Since the products and preparations made from medicinal plants are meant for human consumption, much harped chemical fungicides may have limitations in use due to their adverse effect on the properties of medicinal plants and because of their residual effect and persistence in the system. However, use of fungicides is being discouraged due to economic reasons and growing concern for environment and safety issues.

1.5.2 Cultural Control Practices

Cultural diseases management practices are the measures undertaken to prevent and control diseases by manipulating plants and cultivation practices. Cultural management can include reducing the amount of initial inoculum, reducing the rate of spread of an established disease, or planting a crop at a site that is not favourable to pathogens because of its altitude, temperature or water availability. Stevens (1960) had discussed the cultural methods of disease control. According to him, these measures involve agricultural cropping, harvesting and storage, tillage, crop rotation, organic amendments, soil management and growing of resistant varieties, etc.

- **Reducing the levels of Inoculum**: Practices that reduce the initial levels of inoculums include selecting appropriate planting materials, destruction of crop residues, elimination of living plants that carry pathogens and crop rotation.
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- **Reducing the rate of spread of disease**: The rate of disease spread can be reduced by controlling the spacing of plants, humidity, moisture levels and amount of sunlight.

- **Tillage**: Tillage practices have indirect effects on the spread of plant pathogens. Tillage can bury pathogens in the top soil deeper where they are likely to cause the disease.

- **Intercropping**: The practice of planting more than one crop in alternating rows or intercropping can reduce diseases by increasing the distance between plants of the same species, and creating a physical barrier between plants of the same species.

  These methods are time consuming and yield differential results. It seems that less use has been made of cultural control and modern agriculture has moved towards less use of organic amendments, tillage, crop rotations and other measures of cultural control practices.

### 1.5.3 Biological Control: Present Status and Future Scope

The biological world is a vast network of living organisms interacting in their natural environment. Microorganisms naturally present in the plant ecosystem will help reduce disease potential or disease damage, but only if they are allowed to grow vigorously (Cook, 1993). In a given ecological niche, the interaction between pathogen and antagonist microorganisms may be symbiotic, mutualistic, competitive or antagonistic. The use of ecologically safe and environmental friendly methods of protecting plants from insect pests and pathogens is gaining importance. As in the present scenario, plant disease control has become heavily dependent on chemical pesticides, which are highly expensive, carcinogenic, non-ecofriendly and recalcitrant leaving some residual toxic effects and thereby polluting the environment. Moreover, continuous usage of chemical pesticides may also lead to the development of resistant pathogenic strains, their accumulation can cause serious ecological and health problems. Considering the cost of chemical pesticides and hazard involved, it is desirable to replace chemical pesticides with materials that possess the following three criteria:

- High specificity against the targeted plant pathogens
- Easy degradability after effective usage
- Low mass production cost
Thus, biological control of plant diseases appears to be an effective and ecofriendly approach which is being practiced world over. Therefore, the control of plant diseases by the use of existing organisms in their natural habitat is an urgently needed component of plant disease management.

Biological control means using microorganisms to suppress plant disease, which offers a powerful alternative to the use of synthetic chemicals. According to Baker and Cook (1974), “Biological control is the reduction of inoculum density of disease producing activities of a pathogen in its active or dormant state by one or more organisms accomplished naturally or through manipulation of environment, host or antagonist.”

In spite of the boom in manufacture of synthetic chemicals (pharmaceuticals), the importance of medicinal plants cannot be underestimated. They are a source of numerous known, potent and effective substances and many more, yet to be discovered. It is absolutely essential to protect them from diseases which might reduce the yield of the active compounds. Biocontrol could prove to be an effective and long term solution to this problem and hence the protective role of antagonistic fungi must be explored.

Thus, biological control is also likely to be more robust than disease control that is based on synthetic chemicals. The complexity of the organismal interactions, the involvement of numerous mechanisms of disease suppression by a single microorganism, and the adaptedness of most biocontrol agents to the environment in which they are used all contribute to the belief that biocontrol will be more durable than synthetic chemicals. Biocontrol have potential to replace chemical pesticides (Mukerji and Garg, 1988), therefore biological control agents should be made more efficient to combat the phytopathogenic fungi. In strategies to promote enhancement knowledge of mechanisms contributing to biological control such as mycoparasitism, hyperparasitism (Fokkema, 1981; Sundheim and Tronsmo, 1988) antibiosis (Blakeman and Fokkema, 1982) and competition for resources and space (Lo, Chaur-Tsuen, 1998; Elad and Freeman, 2002) becomes extremely important.

Fokkema (1996) also emphasized on the efficacy of biological control agents formulations and their applications. Potential of biocontrol agents can be improved by several ways such as through genetic improvement (e.g. *Trichoderma* spp. were
mutated so that they were tolerant to benzimidazole fungicides in order to improve their efficiency), provision of appropriate substrates e.g. (*Trichoderma* embedded in peat bran medium) and providing favourable environment for the activity of biocontrol agents (Baker, 1986). The antagonists of phytopathogenic fungi have been used to control plant diseases and 90 per cent of such applications have been carried out with different strains of *Trichoderma* (Monte, 2001).

Biocontrol microorganisms have also been tested as spray application on foliar diseases, including powdery mildew, downy mildew, blights and leaf spots.

### 1.6 Biological control of plant diseases

Biological control simply means that pathogens are antagonized by the presence, activities or products of other similar or different organisms that they encounter in the plant’s rhizosphere or phyllosphere. Thus, biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control (Tjamos et al., 2010).

Biological approaches for the control of pathogens on aerial surfaces have been reviewed extensively (Andrew, 1992 and Blakeman and Fokkema 1982). During this period, most approaches employed for the biological control of diseases of the aerial plant surfaces have concentrated on the use of a single, empirically-selected biocontrol agent to antagonize a single pathogen. The ability to survive on the plant phylloplane is also a desirable trait for strains of fungal and bacterial antagonists used as biocontrol agents against foliar diseases. Spray applications of strain 1295-22 of *Trichoderma harzianum* has resulted in disease suppressive population’s levels on the leaf (Lo et al., 1997). Fungal leaf diseases can potentially be controlled by antagonists preventing infections or reducing the formation of primary or secondary inoculum. Many naturally occurring microorganisms have been used to control diseases on the aerial surfaces of plants. The more common bacterial species that have been used for the control of diseases in the phyllosphere include *Pseudomonas syringae, P.fluorescense, P.cepacia, Erwinia herbicola, and Bacillus subtilis*. Fungal genera that have been used for the control of airborne diseases include *Trichoderma, Gliocladium, Aspergillus, Penicillium, Ampelomyces, Chaetomium* etc. and the yeasts *Tilletiopsis* and *Sporobolomyces*. Antagonism often is based on nutrient competition,
antibiotic production or hyperparasitism. Different mode of actions of biocontrol-active microorganisms in controlling fungal plant diseases include hyperparasitism, predation, antibiosis, cross-protection, competition for site and nutrient and induced resistance. However, antagonistic microorganism can compete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyperparasitisms.

Mycoparasitism and production of volatile and non-volatile antibiotics are important mechanisms operating in case of *Trichoderma*, besides commercial uses and mass multiplication of the novel biocontrol agent. Moreover, efficient biological control agents often express more than one mode of action for suppressing the plant pathogens. To promote enhancement it is necessary to what attributes can be manipulated to make a biological control more competent (Tripathi *et al*., 2010).

Therefore, a knowledge of mechanisms contributing to biological control becomes essential and important (Baker, 1986). For the choice of a control strategy and the selection of a suitable antagonist, the whole life cycle of a pathogen should be considered. Biocontrol involves the use of naturally occurring nonpathogenic microorganisms that are able to reduce the activity of plant pathogens and thereby suppress diseases.

### 1.7 Biological control - A management strategy on the phylloplane

The surface of leaves, the areas of the plant referred to as the phyllosphere or the phylloplane, have been comprehensively reviewed (Preece and Dickinson, 1971; Dickinson and Preece, 1976; Blakeman, 1981; Fokkema and Heuvel, 1986). Biological control of diseases in the phyllosphere has attracted increasing interest. Numerous isolates with antagonistic activity have been isolated from the phyllosphere (Blakeman and Fokkema, 1982) but only a few are able to perform biological control under natural conditions. Ability to harbour high concentrations of natural antagonists of important plant pathogens on the phyllosphere is useful in biological control of aerial plant pathogens (Stromberg *et al*., 1999, 2000).

Biological components of phylloplane are affected by leaf exudates, fluctuating temperature, relative humidity and free water, atmospheric gases, light and radiations, wind and pollution (Sundin 2002; Lindow, 2006; Lindow and Brandl,
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2003; Whipps et al., 2008). In contrast to bacteria, many fungi can live at lower water availability.

Factors tend to change radically all the time. Leaf surface chemicals are present in the form of leachates, exogenous substances and exudates which also affect the phylloplane microflora. Many plant pathogens are potentially affected by biological control agents (Cook and Baker, 1983).

Successful biocontrol on the phylloplane which has been reported in the literature involves mainly rusts, powdery mildews and diseases caused by the genera of pathogens: *Alternaria*, *Epicoccum*, *Sclerotinia*, *Septoria*, *Drechslera*, etc.

To be successful in controlling the pathogen, the introduced biocontrol agent must compete with the other microorganisms and establish an active population on the phylloplane. The aerial surfaces of plants serve as an entrance site, so an introduced antagonist should, above all, interact with the pathogenic invaders efficiently.

There are indications that phyllosphere microorganisms not only can reduce the development of parasites by direct antagonism but are also able to stimulate host plant resistance to disease. Biological methods are a realistic aim for the control of foliar diseases. Lindow (1985 a) also discussed antagonists for foliar pathogens. But their success will depend on a thorough understanding of the microenvironment of the host surface, with detailed knowledge of the pathogen and its antagonists (Elad, 1990).

An alternative or complementary plant health measure is biological control which is being developed and used against *Botrytis cinerea* in a range of crops (Sutton and Peng 1993 b) and might have value for controlling gray mold in *Cyclamen persicum*. Jindal et al. (1988) tested phylloplane fungi for their antagonistic role on *Phytophthora infestans*. Where the maximum inhibition of the potato blight pathogen *in vitro* was given by *Penicilium aurantiocfriseum* followed by *Fusarium equiseti*.

Biological control of blister blight caused by an obligate parasitic fungus *Exobasidium vexans* was made with formulations based on talc/vermicompost with popular antagonists like *Trichoderma harzianum*, *Gliocladium virens*, *Serratia marcescens*, *Pseudomonas fluorescens* and *Bacillus subtilis* (Premkumar, 2001, 2002). Saprophytic microbes were isolated from wheat phylloplane against four target
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pathogens (Septoria tritici, Alternaria triticiculans, Bipolaris sorokiniana and Drechslera tritici-repentis, respectively). In vitro antagonism was measured as the ability of the saprophytic microorganisms to induce morphological changes of hyphae and spores of the pathogens and to reduce conidial germination. In greenhouse experiments phylloplane micro-organisms are also found to significantly reduce the severity of diseases caused by the wheat pathogens. They may also regulate natural colonization, and thus enhance the possibilities for biocontrol (Perello et al., 2002).

Jaiganesh et al. (2007) screened six bio-protectants viz., Aspergillus clavatus, A.nidulans, A.terreus, Pencillium chrysogenum, P.citrinum, P.minioluteum and Serratia marcescens against Pyricularia oryzae causing blast in rice and found that Serratia marcescens was found very effective against P.oryzae under in vitro conditions. The antagonist S.marcescens survived in the phyllosphere even 80 days after spray and also increasing dose of talc based inoculum when applied on foliage and increased the phyllosphere population of S.marcescens and simultaneously controlled rice blast. Karthikeyan et al. (2008) found that the biological control of onion leaf blight disease by bulb and foliar application of the powder formulation of three antagonists (Pseudomonas fluorescense (pf1), Bacillus subtilis and Trichoderma viride) reduced the disease and enhanced the plant growth.

Phylloplane microflora isolated from Cyclamen persicum such as Penicillium spp., Clonostachys rosea, Trichoderma spp. and Aspergillus spp. were found to antagonize the necrosis of the leaves caused by B.cinerea. Whereas, Fusarium spp. such as Fusarium lateritium, F.sambucinum, F.semitectum and F. chlamydosporum were not used for biofungicide development due to the production of toxic metabolites (Rivera et al., 2009).

Basha et al. (2010) isolated and screened the chilli phylloplane microflora and tested several bacterial and fungal isolates for their antagonistic effect against Colletotrichum gloeosporioides and C.capsici which cause anthracnose. Among the fungal isolates of chilli microflora Aspergillus flavus showed (70.2%) and (54.9%) inhibition of Colletotrichum gloeosporioides and C.capsici followed by Trichoderma virens (Tvs-KSD isolate) and T.pseudokoningii (Tpk-1) showing (69.7%) inhibition of C.gloeosporioides. T.viride (Tv-5 isolate) caused (51.9%) inhibition of C.capsici. Among the bacterial isolates Bacillus S-15 showed (30%) inhibition of C. gloeosporioides and S-9 isolates showed (51.3%) inhibition of C.capsici, respectively.
Biocontrol of *Mycosphaerella fijiensis* the causal organism of Black sigatoka leaf disease in banana was done *in vitro* by utilizing the culture filtrates of the three microorganisms (*Bacillus subtilis*, *Trichoderma asperellum* and *Trichoderma longibrachiatum*) in which culture filtrates of *Bacillus subtilis* inhibited mycelial growth of *M. fijiensis* at concentration of 1.5%, 2.5% and 5%. Whereas, *Trichoderma asperellum* and *Trichoderma longibrachiatum* showed effectivity at concentration of 2.5% and 5%, respectively (Abiala *et al.*, 2010). The use of *Trichoderma* spp. and *Aspergillus niger* as biocontrol agent isolated from phylloplane of mustard offers promise in managing *Alternaria* spp. causing leaf blight of mustard *in vitro* and *in vivo* (Reshu and Khan, 2012).

The surface of aerial plant parts provides a habitat for epiphytic microorganisms, many of which are capable of influencing the growth of pathogens. Biological control on the phylloplane proved to be the suitable platform for the growth of antagonists and their potential in combating foliar diseases of numerous commercial medicinal, aromatic and agricultural plants.

Biological control of plant diseases has been considered a viable alternative method to manage plant diseases and its application must be further encouraged and explored for the betterment of people and environment (Heydari and Pessarakli, 2010).

**1.8 Objectives**

Considering the great socioeconomic and therapeutic importance of medicinal plants the main aim of the present study is to explore the potential of antagonistic fungi for the control of diseases as well as for growth promotion of selected medicinal plants.

The objectives of the present study are -

- To identify biological control agents against foliar pathogens.
- To work out a management strategy for biological control of important foliar diseases of medicinal plants.