Review of Literature
Agriculture was developed at least 10,000 years ago and it has undergone significant developments since the time of the earliest cultivation. Agriculture in India has a significant history. Indian agriculture contributes to 8% global agricultural gross domestic product (GDP) to support 18% of world population on only 9% of world’s arable land and 2.3% of geographical area. Agriculture is the key sector for generating employment opportunities for the vast majority of the population; most of them depend for their livelihoods. It is the pivotal sector for ensuring food and nutritional security, sustainable development and for alleviation of poverty. Pioneering work by agriculture scientists and the efforts of farmers had helped achieve a breakthrough in the agriculture sector in the 1960s, popularly known as the ‘Green Revolution’. High agricultural production and productivity achieved in subsequent years has been the main reason for attaining food security to a large extent. The country has not witnessed any big technological breakthrough in agriculture since then.

In global perspective, the production environment and natural resources are continuously deteriorating. Across the larger part of the world, inadequate attention to agriculture has led to steep rise in food prices and increased food riots. Food crisis has aggravated further because of climate change and diversion of arable lands to urbanization and industrialization. Therefore, conservation of natural resources, maintenance of biological wealth and acceleration of agricultural growth are considered of paramount importance in the present context as well as of the future.

With limited land available for cultivation and concerns about environmental sustainability, growth of agricultural output must come through productivity improvements on the existing land. This can only be achieved in parts by suitable control of losses due to various abiotic and biotic agents summarized in Fig 2.1.

2.1 Deteriorating production environment and growing food demand

The food safety net for each of the over a billion citizens a number that is growing requires enhanced agricultural production and productivity in the form of a Second Green Revolution. The demand for food and processed commodities is increasing due to growing population and rising per capita income. There are projections that demand for
food grains would increase from 192 million tonnes in 2000 to 345 million tonnes in 2030. Hence in the next 20 years, production of food grains needs to be increased at the rate of 5.5 million tonnes annually. Urbanisation has led to a fall in natural resources. Thus, the key challenges would be to develop promising technologies and sustainable resource management options to raise productivity to meet growing food demand in a situation of deteriorating production environment (NAAS, 2009).

![Diagram showing factors limiting agricultural yield](image)

**Figure 2.1:** Factors that governs the agriculture yield worldwide

### 2.2 Climate change and agriculture

Inter-Governmental Panel on Climate Change has projected that by the end of this century, global earth temperature is likely to increase by 1.8 to 4.0°C. This would lead to more frequent hot extremes, floods, droughts, cyclones and recession of glaciers. Dynamics of pests and diseases would be significantly altered. The projected increase in these events will result in greater instability in food production and will threaten farmers’ livelihood security. Producing enough food for increased demand against the changing climate scenario is a challenging task for agricultural research.

### 2.3 Pests in Agriculture

With the advent of agriculture, plant diseases, thousands of insects, fungi, viruses, bacteria, nematodes and other living forms pose potential hazards to crops. The agricultural pests and pathogens known so far include 2,000 species of insects and 800 species of fungi. It has been estimated that around 38-42% loss in the global output is due to insect pests, diseases and weeds, despite use of various plant protection tactics
The loss could have increased to 70% if the pests were left uncontrolled. Similar observations have been made by Pimental (1993) who estimated 40% pre-harvest production loss; the post harvest loss adds another 10-20%.

Owing to the tropical climate, the loss is even higher in the Asian countries (Oerke et al., 1995). In India, the pre-harvest production loss due to insect pests is estimated to be 25% for rice, 5 to 10% for wheat and 30% for pulses (Dhaliwal and Arora, 1993). For cotton, rapeseed-mustard and sugarcane, the loss is estimated at 50, 35 and 20%, respectively. The overall comparative losses of crop yield in India and world are summarized in Table 2.1.

Table 2.1: Loss in crop yield caused by insect pests and fungal pathogens

<table>
<thead>
<tr>
<th>Crop</th>
<th>Loss in Yield</th>
<th>In India (%)</th>
<th>Worldwide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td>30.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td>50.0</td>
<td>26.7</td>
</tr>
<tr>
<td>Millet/Sorghum</td>
<td></td>
<td>30.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Cotton</td>
<td></td>
<td>&gt;40.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Pulses</td>
<td></td>
<td>&gt;35.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Sugarcane</td>
<td></td>
<td>&gt;40.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td>30.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Pest management: current scenario

Among various pest control measures like traditional crop rotation, use of natural enemies of pests and development of resistant varieties, application of the chemical pesticides is the preferred method for pest management. Table 2.2 summarizes some common chemical pesticides with their major chemical groups currently used for protection against pests.
Table 2.2: List of common pesticides.

<table>
<thead>
<tr>
<th>Class of Fungicides</th>
<th>Examples</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic Hydrocarbons (Substituted benzene) chlorophenyls</td>
<td>Quintozene PCNB, Blocker</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td>Thiram</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Ethylenebis Dithiocarbamate</td>
<td>Mancozeb, Maneb</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>Thiophtalimides</td>
<td>Captan</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>Dicarboximide (dicarboxamide)</td>
<td>Vinclozoline</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Copper compounds</td>
<td>Kocide</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Bordeaux mixture</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>CuSO₄·3Cu(OH)₂·3CaSO₄</strong></td>
<td></td>
</tr>
<tr>
<td>Organomercury compounds</td>
<td>Phenyl mercury acetate</td>
<td></td>
</tr>
<tr>
<td>Organotin compounds</td>
<td>Fentin acetate (Triphenyltin acetate)</td>
<td></td>
</tr>
<tr>
<td>Triazoles</td>
<td>Hexaconazole</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------</td>
<td></td>
</tr>
<tr>
<td>Strobilurin</td>
<td>Azoxystrobin</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous organic compounds</td>
<td>Metaxyl</td>
<td></td>
</tr>
<tr>
<td>Herbicides</td>
<td>Examples</td>
<td>Structure</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Phenoxy acetic</td>
<td>2,4- Dichlorophenoxyacetic acid</td>
<td><img src="image" alt="Phenoxy acetic Structure" /></td>
</tr>
<tr>
<td>Chlorotriazines</td>
<td>Atrazine</td>
<td><img src="image" alt="Chlorotriazines Structure" /></td>
</tr>
<tr>
<td>Triazinyl sulfonyleurea herbicides</td>
<td>Metasulfuron</td>
<td><img src="image" alt="Triazinyl sulfonyleurea herbicides Structure" /></td>
</tr>
<tr>
<td>Algaecide</td>
<td>Examples</td>
<td>Structure</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Pyrazole derivatives</td>
<td>Pyrazosulfuron</td>
<td></td>
</tr>
<tr>
<td>Quaternary ammonium herbicides</td>
<td>Paraquat dichloride</td>
<td><img src="image1.png" alt="Paraquat dichloride" /></td>
</tr>
<tr>
<td>n-alkyl-dimethyl benzyl ammonium chloride</td>
<td>Polyquat</td>
<td><img src="image2.png" alt="Polyquat" /></td>
</tr>
<tr>
<td>Copper(II) sulfate</td>
<td>Copper Sulphate</td>
<td><img src="image3.png" alt="Copper Sulphate" /></td>
</tr>
<tr>
<td>Colloidal Silver</td>
<td></td>
<td><img src="image4.png" alt="Colloidal Silver" /></td>
</tr>
<tr>
<td>Insecticides</td>
<td>Examples</td>
<td>Structure</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Chlorinated hydrocarbons</td>
<td>Chloropyiphos</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td></td>
<td>Endosulfan</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>Carbamates</td>
<td>Carbaryl</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>Organophosphates</td>
<td>Thiodicarb</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Thiodicarb" /></td>
<td></td>
</tr>
<tr>
<td>Dichlorvos</td>
<td><img src="image" alt="Dichlorvos" /></td>
<td></td>
</tr>
<tr>
<td>Monocrotophos</td>
<td><img src="image" alt="Monocrotophos" /></td>
<td></td>
</tr>
<tr>
<td>Profenophos</td>
<td><img src="image" alt="Profenophos" /></td>
<td></td>
</tr>
<tr>
<td>Acephate</td>
<td><img src="image" alt="Acephate" /></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Quinalphos</td>
<td>Buprofezin</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td><img src="image1.png" alt="Quinalphos" /></td>
<td><img src="image2.png" alt="Buprofezin" /></td>
</tr>
</tbody>
</table>
2.4 Limitations of chemical control measures

Chemical pesticides and herbicides are the preferred methods for pest management, due to their efficacy and cost effectiveness. Data from Pesticide Manufacturers and Formulators Association of India revealed that the Indian Pesticide market exceeded 3.5 billion dollar by usage in 2009. The total industry reached more than $3.35 billion in global sales in 2009. The following figure shows the different class of pesticides and their consumption in Indian agriculture.

Figure 2.2: Consumption of pesticides in India
(Source:http://www.farmchemicalsinternational.com/magazine/)

However, the increased use of pesticides poses serious environmental hazards and public health concerns (Mancini et al., 2008). Numerous reports have indicated direct exposure to the pesticides can induce allergies and affect various body organs such as liver, kidneys, nervous, respiratory and gastro-intestinal systems. Chronic exposures to pesticides are also causative for diseases such as cancer, genetic mutations, and male sterility. According to a report by WHO/UNEP 1989, though the pesticide-use-intensity is low in the developing countries, most of the pesticide related accidents, such as poisoning and deaths occur there. This is mainly because of the misuse of the pesticides and lack of awareness about precautionary measures during its application (Mencher, 1981). Mohan, (1987) observed a high positive correlation between per hectare pesticide use and physical disabilities in India.

Apart with the above health hazards, their indiscriminate and injudicious application has resulted in the development of problems of the pest resistance, resurgence (pest outbreak), and residue.
A number of insect pests, pathogenic microbes and weeds have become resistant to pesticides intended to control them. Worldwide, about 504 insect pests are reported to have acquired resistance to pesticides. This figure was 7 in 1938. Resistance in the weeds was non-existent before 1970, but now 273 weed species have developed resistance to the herbicides (Pimental et al., 1993). Fungicide resistance is reported in nearly 150 plant pathogens. These are serious obstacles to raising the agricultural productivity. Furthermore, the development of resistance to pesticides by pests particularly in developing nations is a serious matter of concern (De Weger et al., 1995; Gerhardson, 2002).

Repeated applications of higher doses of pesticides to overcome the problem of resurgence have increased the cost of pest control. Their exposure inevitably leads to bioaccumulation, biomagnification and adverse effect on non target organisms. Pesticides residues not only affect the ecosystem but also pollute the atmosphere with risky chemicals, contaminate the water we drink, air we breathe and the food we eat. Residues of the pesticides in food grains, fruits and vegetables, fish, milk, water and soil have often been reported to exceed their acceptable limits which have endangered the health of the consumers (ICMR, 1993). These, besides limiting the pest populations, also adversely affect the populations of the beneficial non target organisms such as insects and the microorganisms, predatory birds and the natural enemies of the insect pests.

These health and environmental hazards associated with the use of chemical pesticides are of primary concern to international organizations and governments. Therefore, many countries have banned or severely restricted the use of several hazardous chemicals, including some pesticides used to suppress plant diseases. For example, methylbromide, an odorless, colorless gas used as agricultural fumigant to control a wide variety of pests throughout the world, has been reported by the U.S. Environmental Protection Agency (EPA) to deplete the stratospheric ozone layer and is classified as a class I ozone-depleting substance and banned worldwide (EPA, 2006).

Natural products are now being considered as an alternative to the arsenal of synthetic compounds currently available. These products contribute not only to overcome new diseases but also provide challenges to the synthetic chemists (Cutler
and Hill, 1994; Dayan et al., 1999). Increasing public concern on environmental issues requires alternative plant protection and pest management systems, which are less dependent on synthetic pesticides and are based on naturally-occurring compounds (Cuthbertson and Murchie, 2005).

2.5 Alternative pest control strategies

After severe setback arising from the detrimental effects associated with the use of conventional chemical pesticides, a safer approach to protect the crops from these highly devastating polyphagous pests is urgently needed. Consequently, efforts to obtain pesticides from natural sources have gained momentum (Cutler 1994, 1999). These products can be relatively broad spectrum, bioefficacious, biodegradable and environmentally safe and can serve as ideal candidates for crop protectants to be used in agriculture (Fawcett and Spencer 1970; Gilbert, 1977).

Among various natural sources, researchers have turned their interest towards the isolation of bioactive molecules from microorganisms. Their metabolites are structurally unique and highly active in comparison to plant metabolites. After substantial research over the past 60 years, about 28,000 natural products have been isolated from microorganisms. More than 10,000 of these compounds have been found to be biologically active and more than 8000 are antibiotic and antitumor agents (Fenical, 1993; Berdy, 2005; Betina, 1983; Gustafson et al., 1983). Lichens, in this context, are also catching the attention of scientists worldwide as a source of bioactive molecules of natural origin.

2.6 Brief account of lichens

Lichens are symbiotic organisms composed of a fungal partner (mycobiont) in association with one or more photosynthetic partners (photobiont). Lichens and their products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world (Richardson 1991). The morphology, physiology and biochemistry of lichens are very different from those of the isolated fungus and alga in culture. Lichens are probably the earliest colonizers of terrestrial habitats on the earth, with fossil records tracing back to 400-600 million years ago (Taylor et al. 1995; Yuan et al. 2005). There are about 300 genera and 18000 species of presently recognised lichens, and they account
for about 20% of all fungi (Galun 1988). DeBary (1831-1888) hypothesized that lichens are symbiotic association (mutualism) between fungi and algae: the symbionts benefit from each other. The photobiont can be green algae, cyanobacteria, or both.

![Mycobiont – Photobiont association in lichen](image)

**Figure 2.3:** Mycobiont – Photobiont association in lichen

This unique symbiotic relationship among such diversified organisms makes them flourish in some of the extreme environmental conditions on earth, they can adapt to extreme temperatures, drought, inundation, salinity, high concentrations of air pollutants, nutrient poor and highly nitrified environments (Müller, 2001; Nash, 2008). Thus, these are found worldwide (covering about 8% of the earth’s surface) and their habitat ranges from arctic to tropical regions, from the plains to the highest mountains and from aquatic to xeric conditions. In addition, both fungal and algal cells in the lichen thallus are known for their ability to survive in space (Sancho et al., 2007). They are able to survive in a particular metabolic state for months, due to the slow rate of metabolism and production of many distinctive bioactive molecules which offer chemical protection to them.

According to Conner (2003), lichens grow slowly because the algal and fungal components of the lichens need to grow at similar rates for their survival. If one among them grows faster than the other, the lichens break down. The fungal component of the lichen is very specific about the alga and usually a species of lichen has the same species of alga. Cyanobacteria are particularly useful for lichens living in nitrogen poor environments because the cyanobacteria can fix nitrogen into a form useable by the lichen. In this mutual association, the fungus provides the alga with a more steady supply of moisture, structure and sun protection (presence of the sunscreen pigments). In return, the alga provides the fungus with food. This
association makes the cell wall of the fungus permeable, so that the carbohydrates produced by alga can be absorbed by the fungal tissue.

2.6.1 Morphology of lichens

Lichens are morphologically completely different from their constituent parts, the alga and the fungi. Lichen morphology is extremely diverse; these organisms come in a fantastic array of colors and can vary in size from very small individuals to large structures The morphology of lichens, which is determined by the mycobiont, can be subdivided into two main categories: (Dayan et al., 2001)

1) **Macrolichens** are relatively large in form and easily visible by naked eyes. They include:

   - Foliose lichens which are two-dimensional leafy structures, typically with an upper and lower side, and are commonly divided into lobes.
   - Fruticose lichens which grow preferentially in wet and humid climates are three-dimensional organisms that stand out from their substrate and are characterized as hair-like, shrubby, moss-like, or pendulous.
   - Squamulose lichens have scales like tiny leaves and sometimes resemble match stick.

2) **Microlichens** include crustose lichens, which have a thallus (flat structure) tightly adhered to the substrate at the lower surface and cannot easily be removed.

2.6.2 Versatile applications of lichens

Lichens have been used in folk medicines for centuries, and their biological activities were recognized by Native Americans, Haitian, Indians, Chinese and Europeans to treat a variety of ailments in their traditional medicines (Dayan and Romagni, 2002). Since Egyptians, lichens were used as dyes, perfumes and remedies in folk medicine (Vartia, 1973). Lichens dyed textile reached considerable economic importance in 18th century in some parts of the world as in the Canary Islands (Muggia et al., 2009). Litmus, a blue coloring matter from lichen fermentation, was used as dye for textile and beverages (Beecken et al., 1961). Also, paper strips
impregnated with litmus, a water extracted dyes from *Roccella* sp., are used as pH indicators in laboratories. Extracts of some species of lichens, like *Evernia prunastri*, are used in cosmetics industry as contents of perfumes (Trease and Evans, 1978).

In a study of two lichen species by Munoz *et al.*, 1981, *Parmelia caperata* and *Umbilicaria sp.* are reported as Chilean traditional medicine. *Ramalina thrausta* is used in Finland for treatment of wounds, athlete’s foot or other skin diseases and taken to relieve sore throat and toothache (Vartia, 1973). Various species of lichen are known for treating dyspepsia, bleeding piles, diabetes, bronchitis, pulmonary tuberculosis, spermatorrhoea and other diseases of the blood and heart (Richardson, 1988). For instance, New Zealand Maori traditionally use long, pendulous species of *Usnea* for nappies and sanitary pads (Perry *et al*., 1999). Also, *Usnea* species have been used in Asia, Africa and Europe for pain relief and fever control (Okuyama *et al*., 1995). *Usnea densirostra*, known as “barba de la piedra” served as a cure for various disorders in Argentina’s folk medicine (Correche *et al*., 1998). *Cetraria islandica* is ancient cough remedy known as “tonicum amarum” accepted as a mucilage drug (Muller, 2001). Thus, the most important and studied application of lichens is the one in traditional medicine for treatment of animals and humans.

![Manifold biological activity of lichens](image)

**Figure 2.4:** Manifold biological activity of lichens

During the last part of the 20th century, numerous studies have established manifold biological activities of lichens (figure 2.5). In a work by Esimone and
Adikwn, 1999a, b; Perry et al., 1999 monitored Ramalina farinacea and 69 species of lichens from New Zealand and showed their inhibitory effect against various Bacillus species such as Pseudomonas, Escherichia coli, Streptococcus, Staphylococcus, Enterococcus, Mycobacterium. Behera et al., (2005) reported that acetone, methanol and petroleum ether extracts of lichen Usnea ghettensis were effective against Bacillus licheniformis, B. megaterium, B. subtilis and Staphylococcus aureus. Also, Karagoz et al., (2009) evaluated antibacterial activity of the aqueous and ethanol extracts of some lichens from Turkey against six pathogenic bacteria namely Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus and Staphylococcus epidermidis. Ethanol extracts showed better antibacterial activity than the aqueus extracts.

Halama and Van Haluwin (2004) reported that acetone extracts of Evernia prunastri and Hypogymnia physodes showed a strong inhibitory effect on the growth of plant pathogenic fungi viz., Phytophthora infestans, Pythium ultimum, and Ustilago maydis. Aqueous, ethanol and ethyl acetate extracts of Alectoria sarmentosa and Cladonia rangiferina were found to have moderate antifungal action against different species of fungi namely Aspergillus flavus, Aspergillus fumigatus, Botrytis cinerea, Candida albicans, Fusarium udum, Mucor mucedo, Paecilomyces variotii, Penicillium purpurescens, Penicillium verrucosum and Trichoderma harzianum (Rankovic’ and Mišic’, 2007). Activity was determined against three gram positive bacteria, Bacillus mycoides, Bacillus subtilis, and Staphylococcus aureus and against three gram negative bacteria Enterobacter cloacae, Escherichia coli and Klebsiella pneumonia. Also, in a subsequent report by Ranković et al., (2007b) the minimal inhibitory concentrations (MICs) for acetone and methanol extracts of the lichens Cladonia furcata, Parmelia caperata, P. pertusa, Hypogymnia physodes, Umbilicaria polyphylla and Umbilicaria cylindrical were determined. The study revealed that the bacterial inhibitions varied within the lichen extract, solvent used for extraction and bacteria tested. Extracts of P. pertusa exhibited braod spectrum antimicrobial activities. Bacillus mycoides and Candida albicans were the most sensitive microbe species tested.

Paudel et al., (2008) reported a target-specific strong antibacterial activity of methanol extracts from Antarctica lichens namely Caloplaca regalis, Caloplaca sp., Lecanora sp., Ramalina terebrata, Stereocaulon alpinum against five clinical microorganisms, two gram positive bacteria, Bacillus subtilis and Staphylococcus...
aureus, two gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* and a fungus, *Candida albicans*.

According to Schmeda Hirschmann et al. (2008) dichloromethane and methanol extracts of *Protousnea poeppigii* exhibited strong antifungal effects against the fungal pathogens, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *T. rubrum*. The extracts were also active against the yeasts *Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* and the filamentous fungi *Aspergillus niger*, *A. flavus* and *A. fumigatus* but with much higher strength. In the same assay, extracts of *Usnea florida* also showed strong antifungal properties.

2.7 Chemical screening of lichens

The first studies on “lichen substances” dates back to the origins of organic chemistry. Wilhelm Zopf, a German chemist (Zopf, 1895; and subsequent publications) provided the initial contributions to lichen chemistry. The first review was published as early as 1858 (Gmelin, 1858). Zopf (1907) published a research work on the chemical, pharmacological, and technical aspects of lichen substances. He described about 150 lichen substances based on their chemical properties. In those days little was known about the chemistry of lichen substances, and it was only in the era between 1920 and 1945 that Asahina and Shibata (1954) succeeded in elucidating the structural features of these compounds (Asahina, 1936; Shibata, 1958, 2000; and citations therein). In the second half of the 20th century, thin layer chromatography became a routine technology, and was broadly applied to study the presence of compounds in diverse lichens. The advent of modern analytical techniques, viz., TLC and HPLC, in the 1960s also contributed significantly towards the identification of a large number of lichen substances. This technical improvement helped taxonomists in classifying lichens, many of which differ only in chemical characters. Among the score of literature, particularly important was the publication of Culberson (1969) on the chemistry of lichen substances, followed by two supplementary volumes (Culberson, 1970; Culberson et al., 1977). The number of known substances from lichens increased to more than 800 (Huneck and Yoshimura, 1996; Huneck, 2001). Approximately 1050 secondary compounds have been identified to date (Stocker-Wörgötter, 2008).

2.8 Types of lichen metabolites and their bio activities
According to Asahina’s “Chemistry of Lichen Substances”, 1973, the lichen substances may be classified into the following groups: Aliphatic lichen substances (includes acids and polyhydric alcohols) and aromatic lichen substances (includes pulvinic acid derivatives, depsides, depsidones, Anthraquinones, xanthone derivatives). Lichens secondary metabolites are usually insoluble in water and can be extracted into organic solvents. They amount to between 0.1% and 10% of the dry weight of the thallus. The following types of metabolites are found:

![Diagram of lichen compounds](image)

**Figure 2.5: Types of lichen compounds**

### 2.8.1 Mononuclear phenolic compounds from lichen

Mononuclear phenolic compounds comprise methyl orsellinate (1) (orsellinic acid), phloroacetophenone (2) and phthalide derivatives (3). The lichen derived mononuclear phenolic compounds such as methyl orsellinate (1a) and ethyl orsellinate (1b) display antibiotic activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* (Ingolfsdottir et al., 1985; Fujikawa et al., 1970). Among other monocyclic phenolic compounds, Methyl hematommate (1c) and Ethyl hematommate (1d) showed activity against *Epidermophyton floccosum*, *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*.
T. mentagrophytes, Verticillium achliae; (Hickey et al., 1990). They are rarely found in lichens and are produced by the partial hydrolysis of depsides.

\[
\begin{align*}
(1a) & \ R=H, \ R' = CH_3 \\
(1b) & \ R=H, \ R' = C_2H_5 \\
(1c) & \ R=CHO, \ R' = CH_3 \\
(1d) & \ R=CHO, \ R' = C_2H_5
\end{align*}
\]

### 2.8.2 Phthalide derivatives

Several phthalide derivatives, like 5, 7-dihydroxy-6- methylphthalide (4a) and 5, 7-dihydroxy-6-formylphthalide (4b), were isolated together with alectorialic acid (3) from the lichens Alectoria nigricans and A. capillaries (Solberg, 1970).

\[
\begin{align*}
(3) & \\
(4a), & \ R=Me \\
(4b), & \ R=CHO
\end{align*}
\]

### 2.8.3a Aliphatic acids

Besides the generally wide-spread fatty acids, many lichens accumulate fatty acids with branched chains and 17, 19, or 20 carbon atoms. Unsaturated acids such as arachidonic acid have also been detected in lichen extracts (Yamamoto and Watanabe, 1974). The prototype of monobasic acids is protolichesterinic acid (5), an aliphatic α-methylene butyrolactone. Protolichesterinic acid is chemically unstable and easily rearranges into its tautomeric form, lichesterinic acid, which has the double bond at the α-carbon within the lactone ring.
2.8.3b Polyhydric alcohol

The high tolerance of lichens at sub zero temperature is because of the osmotic activity of sugar alcohols. Polyols help lichens to maintain their physiological activity by protecting lichen thallus from desiccation at subzero temperatures. Natural concentration of polyols in lichen thalli are species specific and depend on the site of collection (Roser et al., 1992b). Actual amount of these sugar alcohols varies with orientation of the thallus and leaching due to rainfall (Dudley, 1987) Mannitol, ribitol and arabitol are abundant in large quantities in lichen thallus (da silva et al., 1993, chapman et al., 1994).

2.8.4 Pulvinic acid derivatives

Numerous lichens contain yellow- or orange-colored pigments that are composed of two phenylpropane units, in which the C₃ unit of these lichen-derived acids is isopropyl rather than propyl. A study conducted by Foden et al., 1975, derivative of vulpinic acid (7), a methyl ester of the prototype pulvinic acid (6), were evaluated as antiinflammatory agents in the adjuvant arthritis test in rats. Abo-Khatwa et al., (1996) showed that vulpinic acid itself possess antimicrobial activity against aerobic and anaerobic microorganisms (Drechslera rostrata, Alternaria alternata). It induces uncoupling of oxidative phosphorylation (Lauterwein, 1995; Raju and Rao, 1986a, b; Raju et al., 1985; Rao et al., 1989).

Emmerich et al., (1993) and Ahad et al., (1991) have studied the antifeedant activity and acute toxicity of vulpinic acid against the highly resistant polyphagous, herbivorous insect Spodoptera littoralis. The metabolite retarded the growth as well as increased the larval period (delayed the pupation) in a dose-dependent manner. The slug-repelling activity of vulpinic acid is being investigated as a seed-treatment for protecting higher plants. According to Hesbacher et al., (1995) application of lichen metabolites in insects may be utilized for chemical defense.
2.8.5 Hydroxybenzoic acid derivatives

Esters of 4-hydroxybenzoic acid such as methyl paraben (8) are used as preservatives in pharmaceutical preparations. As these agents are rapidly hydrolyzed in vivo to the corresponding acid, which is then conjugated and excreted, their toxicity is generally low (Wilson et al., 1998). Some of the lichen derived benzoic acid derivatives, Divaric acid and divaricatinic acid (9, 10), 2-methoxy-4-hydroxy-3, 6-dimethyl benzoic acid (11) and 2-Hydroxy-4-methoxy-3, 6-dimethyl benzoic acid (12) are shown below.

Schmeda Hirschmann et al., (2007) studied antifungal activity of extracts of Andean lichens Protousnea poepigii and Usnea rigida against Microsporum gypseum, Trichophyton mentagrophytes and T. rubrum. The main constituents, divaricatinic acid, isodivaricatic acid, and 5-propyl resorcinol also displayed activity against these fungi.

2.8.6 Depsides
A distinct class of lichen metabolites is the depsides. These types of compounds are formed by condensation of two or more hydroxybenzoic acids whereby the carboxyl group of one molecule is esterified with a phenolic hydroxyl group of a second molecule. These are two different types, with either an orcinol moiety and with a β-orcinol moiety (Asashina and Shibata, 1954). Of the β-orcinol depsides, atranorin (13), diffractaic acid (14) and barbatic acid (15) are moderate inhibitors of arachidonate 5-lipoxygenase (Kumar and Müller, 1999a, b). Accordingly, novel analogues of lichen depsides were synthesized to improve their activity. Kumar and Müller, (1999a, 2000) found that among the depsides, ethyl 4-O-demethyl barbatate (16) and propyl 4-O-demethyl obtusate (17) turned out to be the most potent inhibitors, with IC₅₀ values in the submicromolar range. Kumar and Müller, (1999c) studied several lichen metabolites; the tridepside gyrophoric acid (18) was a potent inhibitor of the growth of human keratinocytes. The activity may be related to its ability to interfere with electron transport systems (Rojas et al., 2000).

Use of lichen metabolites as potential source of herbicides was studied by Dayan (2001). The depsides barbatic acid (15), lecanorin and the tridepside gyrophoric acid (18) act by interrupting photosynthetic electron transport in isolated chloroplasts (Nash, 1996). Lawrey, (1989) studied antibacterial activity of evernic acid (16a), leprapinic acid derivatives like leprapinic acid glycaminide against gram-positive and gram-negative bacteria (Raju et al., 1985).

In a study by Rankovic´ et al., (2008) isolated Atranorin (from Physcia aipolia), fumarprotocetraric acid (from Cladonia furcata), gyrophoric acid (18) isolated from Umbilicaria polyphylla, lecanoric acid (17a) from Ochrolechia androgyna showed strong antimicrobial effects against six plant and human pathogenic bacteria and ten food spoiling fungi.
Among several halogenated depsides in lichens, chloroatranorin (19), tumidulin (20), wrightin (21), other depsides (23-28) were isolated from *Pseudocyphellarie pickeringii*, (22) isolated from the lichen *Lecanara sulphurella*, 3-chlorodivaricatinic acid (23), 3-chlorostenosporic acid (24), 3-chloroperlatolic acid (25) were isolated from *Thelomma mammosum* and *Dimelaena* spp, are reported. Among them, Diploicin (26) was isolated from an unidentified species by Zopf (1904).
(19)

(20)

(21)

(22)

(23)

(24)

(25) R=n-C₃H₇
(26) R=n-C₅H₁₁
2.8.7 Depsidones

Depsidones have an ether linkage in addition to the ester linkage of the depsides, resulting in a rigid polycyclic system. Therefore, they are based on an 11\(H\)-dibenzo \([b,e]\)[1,4]dioxepin-11-one ring system. This chemical feature was shown to be important for the inhibitory activity of this class of lichen metabolites against HIV-1 integrase (Neamati et al., 1997).

![Chemical structures of depsidones](image)

The first representative of depsidones, diploicin (27), was isolated in 1904 by Zopf from an unidentified species of lichen (Huneck and Santensson, 1969). Subsequent research showed that it is present in Buellia canescens and Lecidea cargaleoides (Culberton, 1963, Bendz et al., 1965a, b). Gangaleoidin (28) was
discovered in *Lecanora gangaleoides* lichen. Pannarin (29) was isolated from the lichens of *Pannaria* genus: *P. lanuginosa*, *P. fulvescens*, and *P. lurida* (Elix et al., 1987a, b), *P. pityrea*, *P. rubiginosa*. Pannarin (29), together with the depside atranorin (13), were shown to possess photoprotector capacity and thus may be useful agents to protect against damaging effects of Ultra Violet light (Fernández et al., 1998a, b).

While the open-chain depsides were generally inactive, the β-orcinol depsidones virensic acid (30) isolated from *Alectoria tortuosa* Merr, its methyl ester granulatine (31) and the orcinol-type depsidones physodic acid (32) was found active in the low micromolar range (Neamati et al., 1997). The lichen of *Caloplaca* sp. is found to include caloploicin (33), the structure of which was confirmed by synthesis (Elix et al., 1986 and Jackman et al., 1979).

### 2.8.8 Anthraquinones, naphthoquinones and related compounds

Anthraquinones are widely distributed in nature, and they are particularly prominent in higher plants and fungi (Thomson, 1987). Also, they are important constituents of lichens (Cohen and Towers, 1995, 1996). Several active anthraquinones have been isolated and characterized from some species of the lichen genus *Xanthoria*. Anthraquinones are of interest as antiviral agents against HIV (Schinazi et al. 1990), in particular, hypericin (38) is of pharmaceutical relevance because of its remarkable antiretroviral activity (Lavie et al., 1989). The derivative 7,7′-dichlorohypericin (39) and the anthraquinone 5,7-dichloroemodin (42) isolated from the lichen *Heterodermia obscurata* (Cohen and Towers, 1995), were shown to exhibit strong inhibitory activity against herpes simplex virus type 1 (Cohen et al., 1996). Partial inactivation of the virus was shown by emodin (39), 7-chloroemodin (40) and 7-chloro-1-O-methylemodin (43). Furthermore, derivatives of emodin and chrysophanol (44), which were found in the lichen *Asashinea chrysantha* (Mishchenko et al., 1980), exhibited anticancer activity against leukemia cells in a dose-dependent manner (Koyama et al., 1989). Emodin (39) and its analogues also exhibit strong herbicidal activity on grasses, causing malformation.
and bleaching in early seedlings. Emodin and physcion was found active against *Bacillus brevis* (Anke et al., 1980a, b)

![Chemical structures](image)

(38) R=H  
(39) R=Cl  
(40) R1=H, R2=H, R3=H  
(41) R1=H, R2=Cl, R3=H  
(42) R1=Cl, R2=Cl, R3=H  
(43) R1=H, R2=Cl, R3=Me  
(44)  
(45) R1=H, R2=OH, R3=H  
(46) R1=Cl, R2=OH, R3=H  
(47) R1=Cl, R2=OH, R3=Cl  
(48) R1=H, R2=H, R3=H  
(49)  
(50)  

The reduced form of anthraquinones, the anthrone class of compounds, have been detected in several lichen species (Huneck, 1971) like the 2-chloro- and 2, 4-dichloro-substituted derivatives of emodin anthrone (46, 47). The antipsoriatic anthrone chrysarobin (48) is the reduced form of chrysophanol (Müller et al., 1994).
The naphthoquinone naphthazarin (49), derivatives of which have been isolated from *Cetraria islandica* (Stepanenko *et al.*, 1997), was found to have cytotoxic activity against human epidermal carcinoma cells (Paull *et al.*, 1976). A dimer of naphthazarin, the highly substituted pentacyclic hybocarpone (46) was isolated from mycobiont cultures derived from the lichen *Lecanora hybocarpa*, was shown to be a potent cytotoxic agent against a murine mastocytoma cell line. Also, the naphthopyrone euplectin (50), a lichen metabolite from *Flavoparmelia euplecta*, exhibited moderate activity in this assay (Ernst-Russell *et al.*, 2000).

### 2.8.9 Epidithiopiperazinediones

Recently, compounds that contain an epidithiopiperazinedione moiety have been identified in lichenized fungi. Several scabrosin esters (51-55) from the lichen *Xanthoparmelia scabrosa* were found to exhibit potent cytotoxic activity against murine P815 mastocytomia cells and human breast MCF7 carcinoma cells (Ernst-Russell *et al.*, 1999a).

![Epidithiopiperazinedione structure](image)

- (51) \( R_1 = \text{Me}, R_2 = \text{Me} \)
- (52) \( R_1 = \text{Me}, R_2 = \text{n-prop} \)
- (53) \( R_1 = \text{n-prop}, R_2 = \text{n-prop} \)
- (54) \( R_1 = \text{Me}, R_2 = \text{n-pent} \)
- (55) \( R_1 = \text{n-prop}, R_2 = \text{n-pent} \)

### 2.8.10 Dibenzofuran derivatives

These compounds are quite unusual in nature and are produced almost exclusively by certain lichen species such as *Cladonia* spp., *Stercoreaulon* spp., *Lepraria* spp., *Haematoma* spp, *Lecidea* spp. and *Roccellina* spp. Usnic acid (56) is the major and most studied metabolite of this group. Usnic acid is known to have broad spectrum activity against viruses, microbes, protozoans, insects and fungi (Ingólfsdóttir, 2002). It also absorbs efficiently in the ultraviolet (UV) range of the
spectrum (Rancan et al., 2002), and therefore probably renders some protection against UV-B radiation (Bjerke et al., 2002; Buffoni Hall et al., 2002).

In a study by Rankovic´ et al., (2008) usnic acid isolated from Flavoparmelia caperata showed strong antimicrobial effects against six plant and human pathogenic bacteria and ten food spoiling fungi. The activity of usnic acid was found to be better than standard streptomycin.

Emmerich et al., (1993) and Ahad et al., (1991) have explored usnic acid against the polyphagous and herbivorous insect Spodoptera littoralis and observed that usnic acid demonstrated acute toxicity and feeding deterrence to insect larvae. It retarded the growth as well as increased the larval period (delayed the pupation) in a dose-dependent manner. Both (±) usnic acid, exhibited strong larvicidal activity against the larvae of the house mosquito Culex pipiens in a dose-dependent manner (Cetin et al., 2008). According to Hesbacher et al., (1995) application of lichen metabolites in insects may be utilized for chemical defense.

One interesting aspect of the allelopathic potential of lichens is related to the ability of (−)-usnic acid, to inhibit carotenoid biosynthesis through the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). The in vitro activity of usnic acid is superior to that of other synthetic inhibitors of this herbicide target site (Dayan, 2001). Gollapudi et al., (1994) isolated a novel antimicrobial dibenzofuranoid lactol (alectosarmentin) from Alectoria sarmentosa, which was found active against Staphylococcus aureus and Mycobacterium smegmati.
Synthesis of usnic acid derivatives

The many functional groups of usnic acid make the molecule a good target for structural modification. The compound reacts with amines, hydrazines and acyl hydrazides to form condensation products, undergoes esterification, gives numerous degradation derivatives and forms dihydrousnic acid on reduction. Usnic acid salts are usually unstable. A large number of studies from 1962 to 1985 by the work of Japanese researchers on the chemistry of usnic acid and reaction mechanisms were conducted (Takani and Takahashi, 1985).

Erba et al., (1998) synthesized dipropionate derivative of usnic acid to develop new derivatives for pharmacological evaluation. Preparation of new esters was investigated starting from \( R^- \) usnic acid. Only the monoacetate and the diacetate esters (57a) of usnic acid have been described and prepared by reaction of usnic acid (56) with acetic anhydride, in the presence of an acidic catalyst. By a similar procedure, dipropionate was prepared (57b) in a 60% yield. A better yield of 2b (80%) was obtained by using propionyl chloride and pyridine as acylating reagents. Similarly, starting from chloroacetyl chloride, compound 57c was produced in 82% yield.

![Chemical structure of usnic acid derivatives](image)

\[
\text{57a: } R=\text{CH}_3 \\
\text{57b: } R=\text{C}_2\text{H}_5 \\
\text{57c: } R=\text{CH}_2\text{Cl}
\]

Derivatization aimed at obtaining enhanced biological activity has been attempted by many workers. Shibata et al., (1948) found that antitubercular activity decreased with acetylation of the two hydroxyls in ring A as well as with dihydro-usnic acid.
2.8.11 Macrolides

Macrolides (large ring compounds), representing aliphatic polyketides, are often formed from 9 units (acetyl-CoA and 8 units of malonyl-CoA). So far, many different macrolides of various complexities have been found in prokaryotic and eukaryotic organisms, and recently a new macrolide was found in lichens. Dasypogalactone (59) is unusual in being a 24 membered trilactide, named after the lichen Usnea dasypoga (now Usnea filipendula) and can be recognised as white spots after spraying with sulfuric acid in TLC analyses, and crystallises as white needles. (Suwarso et al., 1999)
2.9  **Advanced studies on lichens**

Lichen studies are primarily limited due to the difficulties observed in their occurrence in extreme habitats and because of their slow growth. In recent decades, improvement in tissue culture conditions for lichen forming fungi has achieved a remarkable progress which has evaded the difficulties encountered with collecting substantial amounts of lichen materials from nature. Ahmadjian, (1974) for the first time suggested the way for isolation of mycobiont using ascospores discharged from the fruit bodies. Since then, spore isolations were conducted by many researchers with different purposes (Ahmadjian and Jacobs, 1987; Stocker-Wörgötter and Türk, 1991, 1993; Culberson et al., 1992; Ahmadjian, 1993; Honegger et al., 1993, Crittenden et al. 1995, Stocker-Wörgötter, 1995, 1997, 1998; Molina and Crespo, 2000; Stocker-Wörgötter and Elix, 2002). In contrast to the fast growth of non-lichen fungi in culture, slow growth of lichen fungi renders difficult to study them as axenic cultures. However, later, this was circumvented by Yamamoto et al., (1985) who introduced a faster ‘tissue culture-method’ for the regeneration of lichen fragments that enables larger amounts of lichen material to be formed within a shorter period of time. However, it should be noted that lichen metabolites produced in mycobiontic cultures are not always identical to those produced from lichens themselves (Brunauer et al., 2007; Yoshimura et al., 1994, Muller, 2001). Mycobionts grown without their photobionts synthesize specific secondary lichen compounds under certain conditions (Culberson and Armaleo, 1992; Fazio et al., 2007; Hager et al., 2008; Mattsson 1994; Stocker-Wörgötter and Elix, 200. Consequently, the number of lichen compounds is much higher than that found in previous literature sources (Culberson and Elix, 1989; Elix, 1996; Elix and Stocker-Wörgötter, 2008; Galun and ShomerIlan, 1988; Huneck, 1999; Huneck and Yoshimura 1996; Lumbsch, 1998). The large increase is due to the fact that, previously, only “natural” substances occurring in intact lichen thalli were counted, but now, substances identified from cultures are also being included.

2.10  **Distribution pattern of lichen metabolites**

It has been shown that the distribution pattern of secondary metabolites is greatly affected by varying conditions of climatic, availability of nutrients, substrates, pH, temperature, light, humidity, stress, topography and varied altitude of the area. The first detailed field survey of the spatial variability of levels of lichen secondary
metabolites (usnic acid levels) with varying climatic conditions was reported by Bjerke et al., (2004). The study showed that the usnic acid concentrations in the arctic lichen Flavocetraria nivalis vary considerably between sites along local longitudinal and altitudinal transects, and the variability is correlated with diverge climatic factors, with the coldest sites having the highest concentrations of metabolites. Thus, the trend towards higher concentrations of usnic acid in colder environments may be a protective response against freezing stress (Kappen et al., 1996; Avalos and Vicente, 1987; Longton, 1988; Rikken, 1995; Huneck, 1999). The study confirmed that the usnic acid levels are altered by numerous stimuli, among which low temperatures and humidity are important factors for usnic acid synthesis. As for usnic acid, there may be some abiotic or biotic differences on microscale causing the high variability. Previous studies along spatial gradients in the field have included comparatively fewer sites with lower control of environmental variables, and have therefore not been able to detect such relationships. The positive correlations with altitude found in some lichen studies was considered as an effect of higher Ultra violet radiation fluxes at high altitudes (Fernández et al., 1998; Quilhot et al., 1998; Bjerke et al., 2002; Rubio et al., 2002). The above study however contradicts this idea and suggests the production of usnic acid may be instead related to the lower temperatures at higher altitudes.

2.11 Descriptive details of the test lichens and test organisms

A detailed survey of literature revealed, that despite, manifold pharmaceutical activities of lichens, studies on potential of lichen metabolites as agrochemicals are scarce. It is also revealed that metabolites production is dependent on certain parameters like the extreme environmental conditions, high altitudes, extreme temperature and varied climate. In addition, the Indian Himalayan region is well known for the richness and diversity of valuable but highly sensitive species of lichens (Kala, 2009). So the present work was undertaken aiming at the survey and collection of lichens from the under collected habitats of higher ridges of Himalayas. These lichens are found upto an altitude of 1000-2000 m in the vicinity of various forest ranges. For the present work, four lichen species namely Parmelia reticulata Tayl., Ramalina roesleri Nyl., Usnea longissima Ach. Articus
and *Stereocaulon himalayense* Lamb were collected from Kilberi forest ranges (Northern Himalayas) in the Uttarakhand State (Uttarakhand). These species are typically found in extreme stressful environment where any other forms of life can’t exist.

**Parmelia reticulata (capera)** is a medium to large, loosely adnate foliose lichen that has a very distinctive pale yellow green upper cortex when dry. The rounded lobes, measuring 3–8 mm (0.1–0.3 in) wide, usually has patches of granular soredia arising from pustules. The lobes of the thallus may be smooth, but quite often have a wrinkled appearance especially in older specimens. The lower surface is black except for a brown margin; rhizines attached to the lower surface are black and unbranched.

**Ramalina roesleri** is characterised by fruticose thalli, the lichen genus Ramalina Ach (Ascomycetes; Lecanorales, Ramalinaceae) (Hale, 1983). There are about 200 species of Ramalina worldwide (kirk et al., 2008) In Ramalina, the thalli, attached to the substrate by a basal holdfast that are erect, tufted (Kashiwadani and Kalb, 1993), and irregularly branched. Ramalina grow preferentially in humid climates and its stand out from the substrate and appear as hair-like, shrubby moss. Fresh thalli of Ramalina are usually gray, greenish gray to yellowish-gray to dark brown when dried. Their branches are much closer to the true branches of plants, however unlike most plants; the lichen branch has no specialized vascular system for transporting fluids (Dayan et al., 2001).

**Usnea longissima** (now Dolichousnea longissima) is fruticose lichen with very long stems and short side branches. It is considered as the longest lichen in the world. It is generally found hanging from the tree branches, resembling grey, greenish hair. It’s also called as old man’s beared. The most common host of this lichen is spruce trees. It is currently comes under threatened species (Source: www.wikipedia.org/wiki/dolichousnea_longissima).

**Stereocaulon himalayense** is characterized by an upright growth of primary thallus, which is called as ‘pseudopodetium’ and the genus was segregated into its own family Stereocaulaceae. The pseudopodetia attached to the substratum (rocks)
usually are erect and simple or branched. Abundant apothecia (reproductive bodies of lichen thallus) can be seen lateral or terminal to the lichen that is more or less rounded and brown coloured.

The solvent extracts and isolated metabolites of these lichens were evaluated for their agrochemical potential against following test organisms:

**Plant Pathogenic fungi**

Fungal phytopathogens pose serious problems worldwide in the cultivation of economically important plants, especially in the subtropical and tropical regions and thereof pose threat for global food security (Brimner and Boland, 2003; Strange and Scott, 2005). Phytopathogenic fungi are difficult to control because their populations are variable in time, space and genotype. Among various fungal phytopathogens soil borne fungi such as *Rhizoctonia bataticola*, *R. solani*, *Fusarium udum*, *Pythium aphanidermatum*, *P. debaryanum* and *Sclerotium rolfsii* are the main pathogens responsible for the diseases and causes high yield losses (Table 2.3) (Park *et al.*, 2005; Pereira *et al.*, 2007; Shenoy *et al.*, 2007; Soares and Barreto, 2008; Than *et al.*, 2008a, b).

In addition, most of them produce mycotoxins, which are harmful to humans and livestock. To a certain extent, pathogens can be controlled by use of suppressive soil, crop rotation and removal of potential inocula, but all these have a limited effect. Alteration in agricultural practices, such as monoculture cropping and the use of synthetic fertilizers and pesticides, have led to a decline in soil structure and an increase in soil borne plant diseases (Bailey and Lazarovits, 2003). The soil-borne pathogens can rapidly colonize even in pasteurized soil and can invade crops through seeds, roots, stalks and cause substantial losses (Wheeler and Rush, 2001; Stephens *et al.*, 1983). Table 2.2 summarizes diseases caused by some important fungal pathogens on various crops. Therefore, these fungi were chosen for evaluating the antifungal potential of lichens species under the study.
Table 2.3: Major phytopathogens and the diseases caused

<table>
<thead>
<tr>
<th>Phytopathogen</th>
<th>Major crop host</th>
<th>Disease caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium udum</td>
<td>Pigeonpea</td>
<td>Fusarium wilt</td>
</tr>
<tr>
<td>Pythium debaryanum</td>
<td>Tomato, Onion, Peas, Cabbage, Chickpea</td>
<td>Foot rot/damping-off, Root rot, Damping off, Powdery mildew, Seedling blight</td>
</tr>
<tr>
<td>Rhizoctonia bataticola</td>
<td>Groundnut/oil seed crops, Cotton, Pigeonpea</td>
<td>Dry root rot/dry wilt Root rot</td>
</tr>
<tr>
<td>Rhizoctonia solanii</td>
<td>Potato</td>
<td>Rhizoctonia stem lesion</td>
</tr>
<tr>
<td>Sclerotium rolfsii</td>
<td>Lentil, Groundnut, Onion, Gram, Rice (nursery bed)</td>
<td>Collar rot, Stem rot Sclerotium wilt, White rot, Collar rot of gram, Seedling blight</td>
</tr>
</tbody>
</table>

Weeds

Weeds are the next main agricultural yield limiting factors in most parts of the world. Weeds are any undesirable plants grown in field along with the main crop plant. In particular, Phalaris minor Retz has been identified as a serious weed of wheat and barley fields in India, USA, Canada, Africa, Australia, France, Pakistan, Iran, Iraq and Mexico (Holm et al., 1979; Shad and Siddiqui, 1996). Furthermore, a rice wheat crop rotation also stimulated its emergence, growth and development (Chhokar and Malik, 1999). Weed seeds of many weed species may be killed during the summer or by rainy season flooding under rice cultivation, P. minor remains unaffected due to an impermeable seed coat. The weed is highly competitive and can cause significant yield losses under favourable conditions. Depending upon density of P. minor, 10-65% yield losses (in India) in wheat have been reported by various workers (Mehra and Gill, 1988; Dhalival et al., 1997; Dhima and Eleftherohorinos, 2003; Chhokar et al., 2008). Due to morphological similarity of P. minor with wheat, manual weeding at early stages is difficult.

Microalgae

Microalgae are the first algae emerged over 2 billion years ago and first to carry out photosynthesis and converted earth’s oxidizing atmosphere into reducing one. Algae are of particular interest for studies because they inhabit wide range of
ecological habitats. They are well endowed with effective protective mechanisms against different biotic stresses and have remarkable adaptability to varying environmental conditions (Jabir, 2009). Another major global aspect is determining the effects of these algae in natural fresh water ecosystem, because of the continuing widespread growth of them. Although algae don’t influence the agriculture directly but may hinder the agricultural yield in many indirect ways. By covering the water surface, they alter the life cycle of aquatic flora and fauna, also, disturbs the irrigation requirements of any field.

Microalgae used in the study namely *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* belongs to the chlorophyceae family of algae. It’s a large and important group of freshwater green algae. The Chlorophyceae are one of the classes of green algae, distinguished mainly on the basis of ultrastructural morphology. Chlorophyceae algae include some of the ecologically important species of algae like *Chlorella vulgaris* and *Chlorella sorokiniana*. *Chlorella* is a genus of single cell green algae, belonging to the phylum ‘Chlorophyta’. It is spherical in shape, about 2 to 10 µm in diameter, and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll a and b in its chloroplast. It requires only carbon dioxide, water, sunlight and a small amount of minerals to reproduce. *Scenedesmus* is another genus of the chlorophyceae group. These are usually colonial and non motile. These green algae usually have a rigid cell wall of an inner layer of cellulose and outer layer of pectose.