Chapter 1

INTRODUCTION
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From the pre-historic age, man had the knowledge of plants, which he used as diet and drug. There are about 1.5 million plant species existing on the earth. About 8000 of these species are used as traditional medicines. Out of which only 1% of these are acknowledged through scientific studies.

Plants are susceptible to one or other forms of life leading to unwanted changes in molecular levels which leads to a high range of diversity among them. Therefore the plants of agricultural values are infected by biological forms like viruses, bacteria, fungi, and nematodes.

To control these biological forms pesticide compounds are playing a vital role in controlling the diseases caused and on contrary increasing the agricultural production. The practice through which specific plant species are cared and managed so as to obtain maximum yield of consumable parts of the plant. An agriculture field is, in fact, a cropland ecosystem in which ecological principles involving limiting factors and productivity provide great applicability.

The future role of pesticide in agriculture is increasingly threatened by several factors including the development of pest resistance, increase concerns about food safety and environmental accumulation of toxic compounds. As older pesticides are removed from the market due to regulatory changes and new pesticides are becoming increasingly expensive to register, there is an increasing need to find ways to more wisely use the remaining, safest pesticides. This is particularly true for the many crop/disease combinations which do not represent large enough markets to pay for the cost of new compound registration. Wiser pesticide use will include ways to reduce application rates, finding ways to extend registrations to new crops, and identifying new compositions and treatments to
combat the development of pest resistance. However fungicides cause health effects particularly when used inadequately. The most consistent adverse effect is allergic dermatitis and skin or mucous membrane irritation, etc. In addition to decreased crop yield fungi are responsible for the production of mycotoxins occur in wide variety of substances, including food and feeds and can cause both economic and health losses. The use of fungicides and unopposed growth of fungi represent potential health hazard.

Pesticide have provided an effective method of control, however, the public has become concerned about the amount of the residual chemicals which might be found in food, groundwater and the environment. Stringent new restrictions on the use of chemicals and the elimination of some effective pesticide from the market place could limit economical and effective options for controlling pests. In addition, the regular use of chemical toxins to control unwanted organisms can select for resistant strains. Alternative strategies to pesticide application are needed for the control of agriculturally important pests. Such strategies will help address public concern regarding pesticide pollution.

Agriculture industry has faced problems arising from the development of resistance in fungal pathogens of crops against use to control them. At the present time, when sustainability, economic, technical, environmental are becoming the primary aim of modern agriculture, it seems appropriate to make a broad review of progress world wide in dealing with fungicide resistance, and the outstanding difficulties which need to overcome. Use of chemical fungicides in the management of diseases like soil drench or paint on the wounded plant surface, spraying or dusting has been suggested by many workers. Any single approach of control these doses do not help in reducing the disease and requires multiple strategies methods like use of culture practices, biological control agents, fungicides and host resistance.
The last problem of fungicide resistance was first raised by Horsfall in his book, ‘Principles of fungicidal action’ in 1956. He had warned regarding the possibility of development of resistance by fungi to specialized fungicides. However, there were few incidences of fungicidal resistance in fungal pathogens when Georgopoulos and Zaracovities (1967) reviewed tolerance of fungi to organic fungicides after 21 years. In 1970’s many articles appeared in journals especially one by Prof. Dekker (1972) in systemic fungicides. Ogawa et al 1976 summarized field resistance in plant pathogens to certain chemicals. American Phytopathological Society also held a symposium on resistance of plant pathogen to chemicals in 1976. Chemical control news letter reports fungicides resistance cases since 1983 published by International Society of Plant Pathology (ISPP). A fungicide resistance action committee (FRAC) is also framed for this purpose. Attention towards fungicide resistance is also focused by Gangawane (1981, 2000) in India. Literature survey by Reddy (1986) indicated that nearly 72 fungal species are reported to be resistant to 62 fungicides all over world. Maximum cases have been reported in US, followed by Japan and European countries.

In India control of many pathogenic fungi with systemic and non- systemic fungicides has become more and more common over past 20 years. Presently more than 140 compounds have been registered and sold in different formulation. Fungicide resistance is registered in many fungi such as Benomyl, Captafol, Captan, Carbendazim, Chlorothalonil, Quintozenes, Maneb, Mancozeb, Thiophanate methyl, Thiram, Ziram etc. Fungicide resistance is registered in Aspergillus flavus to Captafol, Quintozenes, Maneb, Thiophanate methyl Captan and Carbendazim (Gangawane and Saler, 1982; Gangawane and Ramchandra Reddy 1995, Reddy 1995). Carbendazim resistance management was studied by Chander, M and Thind T.S. 1995, Gangawane et al 2000. Brent JK, 1995 showed fungicide resistance in crop pathogens. Alternaria solani to Carbendazim and Thiophanate methyl (Gangawane and Sahera Nasreen, 1992) Fusarium oxysporum f. sp. Lycopersici to Mancozeb and Ziram (Dhabadkar
S D and Sahera Nasreen, 2002) Colletotrichum capsici to Mancozeb and Ziram (Dehade Jitendra and Sahera Nasreen, 2006). Pathania, A., P.N. Sharma, O.P. Sharma, R.K. Chahota, B. Ahmad, and P. Sharma. 2006. Evaluated resistance sources and inheritance of resistance in kidney bean to Indian virulence of Colletotrichum lindemuthianum. Factors such as plant productivity, diseases affect the quality, quantity and availability of food throughout the world. So improvement in the diagnosis and management of plant disease are a priority in such instances. Studies are concerned with practical solution to the problem of mung bean disease. Studies are based on mixture of pure and applied aspects of biology. A distinction is often drawn between disease caused by infectious agents and disorders due to non – infectious agents such as mineral deficiency, chemical pollutants or adverse climatic factors. Emphasis of this study is on the disease caused by fungi under favorable condition; these pathogens can multiply and spread rapidly through plant population to cause destructive disease epidemics.

A fundamental concept in this work shows that, disease results from an interaction between the host, pathogen and the environment which can be altered or reduced by various methods.

The mechanism by which fungi C. lindemuthianum cause disease (Fungal blight) is governed by a series of events that begins with the adhesion of fungal spores to host surface tissue, followed by spore germination, appressoria formation, and penetration into the first subcuticular cell (Bailey et al., 1992). If a compatible interaction takes place, the pathogen will exhibit necrotrophic characteristics and quickly disseminate through host tissues, ultimately resulting in necrotic lesions and causing plant disease. In an incompatible interaction, a rapid, localized collapse of tissue surrounding the initial infection zone occurs, resulting in disease resistance. The resistant reaction, designated the hypersensitive response, is believed to be genetically programmed and bring together disease
resistance due to the recognition and interaction of biochemical components from both the pathogen and host. (Flor, 1971; Keen, 1982, 1986, 1990; Klement, 1982; Kuc, 1990; Alfano and Collmer, 1996; Hammond-Kosack and Jones, 1996; Jackson and Taylor, 1996; Knogge, 1996). In compatible interactions, widespread plant cell death may occur because of the delayed response of the plant to the presence of the pathogen and the ability of the pathogen to overcome the plant host-response system (Darvill and Albersheim, 1984; Davis et al., 1986; Ebel, 1986; Bailey et al., 1992; Jackson and Taylor, 1996).

Susceptibility or resistance to disease by *C. lindemuthianum* or other plant pathogens appears to follow a common theme involving the temporal and spatial expression of plant-defense components activated by a number of fungal and/or plant metabolites (Anderson, 1978; Ebel, 1986; Hamdan and Dixon, 1986; Kombrink and Hahlbrock, 1986; Anderson, 1988; Dixon and Lamb, 1990; Bailey et al., 1992; Nicholson and Hammerschmidt, 1992; Knogge, 1996., Kuc 1990; Kuc and Strobel (1992) implied that susceptible cultivars may be manipulated to resist pathogen attack by altering the timing and magnitude of the defense response.

An additional dimension to incompatible interactions involves the extent and spatial distribution of the plant-defense response resulting in a rapid, localized, and/or systemic form of protection. A localized response occurs around the site of pathogen entering site and protection is afforded to the plant cells in the surrounding area. Often, a small, necrotic lesion will be formed as a result of the hypersensitive response.

Key components postulated to play important roles in localized resistance include increased activity in peroxidase and deposition of lignin in tissues. Peroxidase is involved in cross-linking extension molecules and in the
polymerization of hydroxycinnamyl alcohols to form lignin. (Hammerschmidt et al., 1982; Dalisay and Kuc, 1995a, 1995b). Increased lignin deposition is believed to play a role in barricading the pathogen from invading the plant through physical exclusion (Hammerschmidt and Kuc, 1982a, 1982b; Hammerschmidt et al., 1984; Nicholson and Hammerschmidt, 1992; Hammond-Kosack and Jones, 1996).

Fungal blight disease caused in mung bean (*Phaseolus aureus*) is caused by the fungus *C. lindemuthianum* which severely reduce seed yield, quality brand marketability in all the major bean classes.

The fungal blight fungus causes dark brown lesions to form on all the above ground parts of the plants. On the leaves, lesions typically occur along the veins and are most obvious from the lower side of the leaf. Heavy infection of the leaves often results in early defoliation. Fungal blight also causes the formation of sunken lesions on the stem and pods. Pod infection ultimately results in the formation of dark brown lesions on the seed.

Studies on fungicidal test on *C. lindemuthianum*, causing fungal blight of Mung bean were undertaken. It was observed that there was variation between 20 isolates obtained from the field. Some isolates showed profuse growth about two folds suggesting the possibility of resistance to fungicide. Therefore studies on the fungicidal resistance in *C. lindemuthianum*, and management of fungal blight disease were undertaken and it is presented in the thesis.

The fungicides which were selected for the study are Carbendazim, Benomyl, Thiophanate methyl, Mancozeb and Chlorothalonil. All these fungicide having systemic and non-systemic mode of action are used to control the diseases
of *C. lindemuthianum*, causing fungal blight disease of Mung bean. Carben dazim and Mancozeb were selected for further studies.

Antifungal activities of leaf extracts of 10 plant species have been studied. Extracts of plants used were *Adhatoda vasica*, *Azadirachta indica*, *Catharanthus roseus*, *Calotropis procera*, *Casuarina equisetifolia*, *Hyptis suaveolens*, *Eugenia jambos*, *Lantana camera*, *Ocimum sanctum* and *Tridex procumbens*.

Bulb extracts of *Allium cepa*, *Allium sativum*, and rhizome extracts of *Zingibar officinale* were also tested against pathogen.

Biological control potential against *C. lindemuthianum* was also evaluated. The antagonistic potential for five fungi and two bacteria was assessed against the test organism *i.e.* *C. lindemuthianum*. The fungi used were *Aspergillus flavus*, *A. niger*, *Gliocladium virens*, *Penicillium aurantiogriseum* and *Trichoderma viride*. *Bacillus subtilis* and *Pseudomonas fluorescens* was used as bacterial source against the test organism.

The resistance is used for each form of decreased sensitivity of a pathogen to fungicide. However the tests were carried out to see whether resistance is permanent or temporary on media.

Sensitivity of twenty isolates of *C. lindemuthianum* against fungicides was evaluated. On the basis of which isolate *Cl*-11 was considered to be resistant isolate while *Cl*-19 was considered to be sensitive isolate. Latent period is very important for successful infection and development of the symptoms and simultaneously latent period of the pathogen was also recorded. Fungicides application programme may influence the development of resistance in the pathogen. Hence effect of successive passages of *C. lindemuthianum* on mancozeb and carbendazim individually, alternately or in mixture with other fungicides with
different mode of action was studied both *in vitro* and *in vivo*. Due to altered metabolism in the pathogen the resistance may increase or decrease the virulence of the pathogen.

A comparison of certain physiological and biochemical characteristic of resistant and sensitive isolates was also made. It was observed that growth rate of resistant isolate was higher than that of sensitive isolate.

Agrochemical other than fungicide such as antibiotics, herbicides, fertilizers, micronutrients and salts were used in the crop disease management. These chemicals had effect on the development of resistance in the pathogen. Many of the chemicals broke the resistance or vice-versa when in combination. Effect of temperature and pH on resistance was also studied *in vitro*.

Survival ability of resistant isolates using the different proportions of resistant and sensitive populations and in the population of other dominant microfungi was noted. This gave the fitness or resistance in the *C. lindemuthianum* in nature.