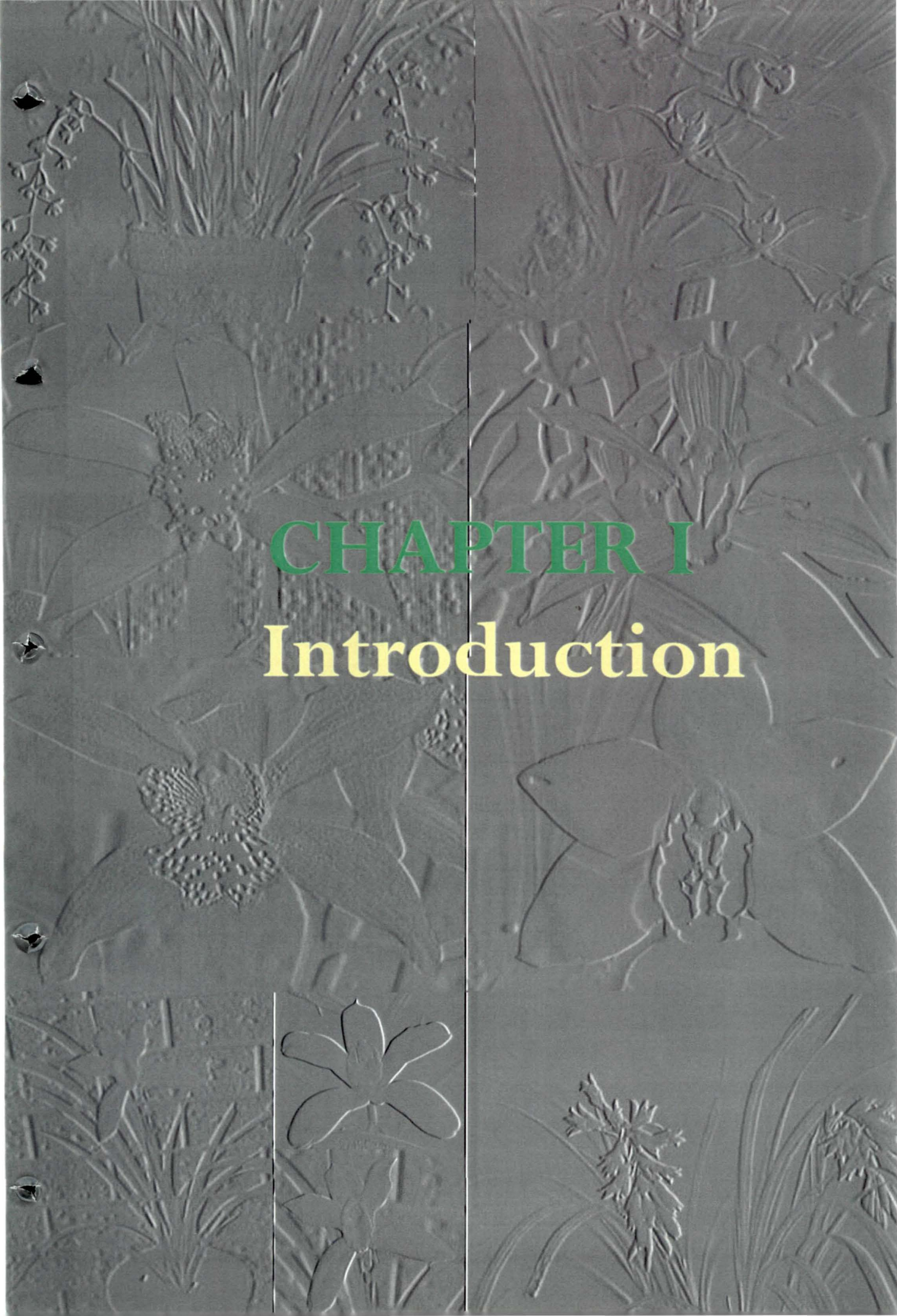

CHAPTER I

Introduction

1.1. ORCHID: AN OVERVIEW

Theophrastus, 'the father of botany' (370-285 BC), gave the name "ORCHIDS" to a unique group of plants bearing the most beautiful flowers of nature. Taxonomically, they represent the most highly evolved family among monocotyledons with more than 800 genera and over 25,000 species that are commercially grown all over the world (Arditti, 1992). Orchids exhibit an incredible range of diversity in size, shape and colour of their flowers. They are most pampered of the plants and occupy top position among all the flowering plants valued for cut flower production and as potted plants. In India they are known for their longer lasting and bewitchingly beautiful flowers which fetch a very high price in the international market. The morphological, physiological, and genetic peculiarities inherent in this group of plants have stimulated research to such a degree that Orchidology today is one of the most popular and dynamic branches of Botany (Pathak *et al.*, 2001). Besides their unmatched ornamental values, orchids have some value in traditional medicines also (Srivastava, 1999). The drug obtained from the roots of *Cypripedium pubescens* is useful in the treatment of stomach worms and in allaying pains of the joints (Lewis and Elvin-Lewis, 1977).

In the Indian Vedic scriptures there is a mention of the plants under the name "VANDA", which has been adapted as a generic name in one of the most beautiful group of orchids. Most of the orchids are perennial herbs with simple leaves. Although the specialized flower structure conforms to a standard formula, the vegetative part shows great variation. Large number of them being epiphytes, or terrestrial and a few are saprophytes and leafless in nature. Majority of the cultivated orchids are native of tropical countries and occur in greatest diversity in humid tropical forest of South and Central America, Mexico, India, Ceylon, Burma, South China, Thailand, Malaysia, Philippines, New Guinea and Australia. Brazilian *Cattleya*, Mexican *Laelia* and Indian *Dendrobium*, *Cymbidium* and *Vanda* have played a major role in the development of modern orchid industry in the world (www.orchidsasia.com/orcintro.htm).

The major developments in cultivation of orchids in the world have been due to modern scientific technologies, which have been suitably used in case of orchid seed germination and meristem culture. Today orchids are grown on assembly-line method in extensive glasshouses with controlled environment and the sale of orchid flowers runs in millions of dollars. The modern methods of propagation have brought orchid cultivation on parity with other commercial crops. Horticulturists worldwide today grow orchids not only because they are mysterious, but mainly due to the fact that they are highly priced and occupy 8% share of the Global floricultural trade (www.sikkim.nic.in/nrco/nrco.htm).

The economic importance of orchid lies mainly in their ornamental value and horticultural uses. They provide cut blooms which remain fresh for long, make pretty corsages and used for long in Europe and the USA as progenitors for the production of some of the famous hybrids and even today are in great demand by orchid dealers abroad (Arora, 1985). The world export/import trade of orchid cut flowers and orchid plants exceeded $150 million in the year 2000. Of this, $128 million was in cut orchid flowers and about $23 million in orchid plants, according to United Nations Comstats. Thailand is the world's largest orchid exporter with 2,240 hectors of orchids in production. Singapore is also an important exporter, with $7.7 million in exports of cut orchids and $8,000 in orchid plants. Malaysia, much smaller, exported $2.8 million in orchid cut flowers and $15,000 in pot plants. New Zealand exported $830,000 in cut orchids and $93,000 in orchid plants. Only one European country Italy, is a significant exporter of orchids, which exported $652,000 in cut orchids and $399,000 in potted plants. The world commerce in orchid cut flowers is eight times as important as the world trade in orchid plants (Laws, 2003).

From a commercial standpoint, the most important orchids are those grown for cut flowers, which include species and hybrids of *Arachnis*, *Aranda*, *Asocentrum*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Laelia*, *Oncidium*, *Paphiopedilum*, *Phalenopsis*, *Renanthera*, and

Vanda. Annually, US$ 35 million worth of orchid cut flowers are produced and exported by Thailand (Zettler *et al.*, 1990). Singapore, being the world's second largest exporter of orchid cut flowers, exports US$ 24 million worth of cut flowers annually (Zettler *et al.*, 1990). *Dendrobium* orchid hybrids, one of the most economically important cut and potted floricultural crops grown in Hawaii, commanded a wholesale value of US$ 6.3 million in 1990 (Hu *et al.*, 1993). With the help of various mass-production methods, orchids account for 23 percent of the total production value of Taiwan's domestic flower industry. The total area of land devoted to orchid growing in Taiwan today is about 460 hectares, with an annual export value estimated at US $45 million (Chu, 2004).

Considering the number of species, India is certainly rich in orchid flora (Jain, 1985). India is not only rich in number of orchid taxa which grow profusely in nature, but most species are at top of the list of ornamental orchids (Sharma, 1996). Orchids form 9% of our flora and are the largest botanical family of higher plants in India. India is home to about 1,700 species of orchids (Varmah and Sahni, 1976), of which about 800 are found in the North Eastern region of the country where North Eastern Himalayas ranks the top in the list. Within these areas, Sikkim Himalaya, comprising the hills of Sikkim and Darjeeling, harbours about 450 species and the centre of origin for important species like *Cymbidium*. Mehra and Vij (1974) studied ecological adaptations and distribution pattern of East Himalayan orchids. Similar data was presented from the Simla (Vij *et al.*, 1982) and Nainital (Vij *et al.*, 1983) hills in the North-Western Himalayas.

India has been mainly exporting native species of orchids. In accordance with the recent Convention of International Trade in Endangered Species (CITES), the trade in native species from wild sources has been banned. India's export of the orchid flowers is yet to reach a sizeable figure. Some reputed sources indicate that the country exports orchid flowers to Europe, Hong Kong, Japan and Australia. However, India's potential

for the export of orchid flowers is rated as high. Though India's present export of orchids is meager, its future potential is high in view of the following reasons:

A large number of orchid species grow naturally in the congenial climate of India. The northeast and southwest regions are considered as two "hot spots" of the orchids. Of the numerous species of orchids only a few can be successfully grown in the plains.

- The climatic conditions may permit the cultivation of the orchids, either in the open or in simple green house, so as to export round the year.
- The cheap labour, land and input cost enable Indian orchids to be cost effective.
- India is strategically located between Middle East, Far East and EEC countries viz. Italy, the Netherlands, Germany, France, U.K. markets.
- Decreasing availability of land area, due to rapid urbanization in the South East Asian countries, which are the major exporters of orchids, is opening up the possibility of shifting the orchid production centers to South Asian countries.
- The recent economic liberalization initiated by the Union Government, and emphasis on the promotion of agri-export and the development of infrastructure have created congenial conditions for setting up of export oriented units of orchids.
- A domestic market for the orchids is fast developing, which can absorb, non-exportable surplus. (www.nabard.org/whats/orchid.htm)

The *Cymbidium*

Many people think first of a *Cymbidium* flower when they hear the word 'orchid'. Cymbidiums are widely grown by orchid enthusiasts throughout the cooler parts of the world and they form the basis of a significant cut-flower production. The flowers are long-lasting, both on the plant and when cut, large, attractive and available in a wide range of colours (Figure 1.1). This popular ornamental genus of the orchids is reported

to have 300 species. *Cymbidium* is truly an Asiatic orchid since it is most widely distributed in Asian countries. The North-East Indian hills are the richest phyto-geographical habitat for this genus in India because of the prevailing supporting climate (Munsi *et al.*, 2004). It thrives well in the temperature ranging from 10^0C (night) to 25^0C (day). Depending on the temperature requirements, there are two broad groups of *Cymbidium*: (a) tropical, which requires higher and (b) temperate, which requires lower temperature. However, most species flowers, when the night temperature is about 10^0C, but it should not be less than 4-5^0C. The *Cymbidium* species in nature are usually tree dwellers (epiphytic) or live on rocks (lithophytic), but some are even ground dwellers (terrestrial). However, this group of orchids is treated as semi-terrestrial in commercial cultivation and grows on pots containing a medium with the characteristics of rapidly drainable yet moist. The Cymbidiums belong to sympodial group of orchids, and can be propagated by the division of rhizomatus clump when the plants have more than 8 pseudobulbs (swollen stems) (DuPuy and Cribb, 1988). Plants which normally grow on trees are sometimes encountered on rocks, and one species, *C. macrorhizon*, is a saprophyte (Hooker, 1890) which grows entirely beneath the soil surface except when the flower spike emerges. Most species have thick roots which are covered in a spongy white velamen and have only a thin core of vascular tissue. The erect stems are usually short and swollen to form a prominent pseudobulb which is often slightly flattened. Many species produce a new growth annually. In one section of the genus the pseudobulbs grow and flower for several years before a new shoot is produced, and in *C. mastersii*, *C. elongatuni* and *C. suave*, each shoot grows continuously for many years producing an elongated stem rather than a typical pseudobulb.

Fifure 1.1: Different species of Cymbidium

1.2. GLOBAL OCCURRENCE OF ORCHID DISEASES: A REVIEW

The orchids are adapted to withstand a variety of environmental stresses but under highly adverse growing condition their natural defense mechanism generally gets weakened and they succumb to a variety of diseases. The susceptibility however, varies with the geographical regions, genera, species, hybrids and even individual clones. Besides the physiological factors, the orchid diseases are usually caused by fungi, bacteria, viruses, algae, weeds, insects etc. the main cause of stress in orchids are related to water. Too much water, due to over watering of poorly drained medium, leads to their rot and subsequent attack by fungi and bacteria. The impaired health status of the diseased plant detrimentally affects their commercial significance and they are outrightedly rejected in the floriculture trade (Vij and Kaur, 1985).

Information on orchid diseases and their control has been accumulating over the years. It can be effectively used to ameliorate the health and commercial status of the diseased plants and is of great interest to professional and amateur orchid growers. It is better to prevent diseases from its initial development and it is possible if a grower uses good cultural practices unfavourable for disease infection or pest attack (Burnett, 1965). Proper sanitation in the greenhouse and application of preventive steps are very important to keep the plants free from various pests and diseases. Various orchid diseases are discussed here:

1.2.1. FUNGAL DISEASES: -

1.2.1.1. Leaf Spot: - This disease is very common and can be found in almost all cultivated species of orchids. It is caused by species of *Gloeosporium* (Alexopoulos, 1935), *Colletotrichum, Phyllostictina*. This disease have been reported from Germany, England and other countries by Pirone *et al.*, (1960). Within a few days of infection sunken spots appear at any place on the leaves which later turn brown. Warm humid weather and lack of light encourage the spread of disease. Spraying of Bordeaux mixture effectively controls the

disease. Yellow oxide of copper, 1lb in 100 gallons of water, also controls the disease if applied regularly to the plants (Ark, 1959).

Another leaf spot disease of orchids was reported from Wutong Shan Orchid Nurseries, Shenzhen, Guangdong, China. The fungus produced spots on orchid leaves in mid-February. The disease epidemic peak occurred from April to June when cloudy and damp weather prevailed. The disease was more serious when plants were cultivated under hot, windless conditions. After applying Koch's postulates and a comparative inoculation test, the pathogen was identified as *Stagonospora curtisii* (Wu *et al.*, 1997).

Cymbidium leaf spot is caused by *Fusarium* species which was consistently isolated from yellow, swollen spots with reddish brown centers and small black spots on leaves of *Cymbidium* plants in the greenhouse. *Fusarium subglutinans* caused the yellow spots and *Fusarium proliferatum* caused either the yellow or the black spots. Kazunori and Takayuki (2000) propose the name "yellow spot" for the new disease.

1.2.1.2. *Pythium* Black rot: -This disease affects mostly seedlings in community pots and is caused by *Pythium ultimum* (Ark and Middleton, 1949). Affected plants turn black and leaves start falling. The pseudobulbs also start to rot within. Since in community pots the plants are closed to each other, the disease spreads from plant to plant at a very rapid rate. Orchid such as *Epidendrum* sp were found to be susceptible to *Pythium ultimum*. Withholding of watering for a few days and shifting the plants to less humid part of the green house help to check the disease. Infected plants should be removed and the remaining healthy ones spread with fungicide like mancozeb @ 2.5 gm/lit. Irrigation with a mild solution of a copper fungicide once a month till the plants are tender, is an effective preventive measure.

1.2.1.3. Heart Rot: - *Cattleya, Phalaenopsis* and *Vanda* are the important orchid genera susceptible to the disease, which is caused by *Phytophthora palmivora*. The leaves of the infected plants turn yellow and drop off. Pseudobulbs have dark rotted areas inside. The infected pseudobulbs should be removed and the plants repotted in fresh media after dipping in fungicide. Gilbert et. al. (1950) tried two different methods of healing orchid plants to control a species of *Phytophthora*. In the first step inoculated *Cattleya* plants were placed in a forced draft type poultry incubated at 107^0F, 90% relative humidity for 24 to 66 hours. All plants showed injury but new healthy growths were produced later. In the second method, inoculated plants were exposed to vapour heat at 116^0 or 120^0 F for 39 min to 1 hour and 25 min. With this treatment orchid plants showed no apparent heat injury but the fungal growth was checked.

1.2.1.4. Brown Speck and Blight of Flowers: - The disease affects a number of orchids like *Cattleya, Phalaenopsis, Dendrobium Onchidium* and *Vanda*. It is caused by *Botrytis cinerea*. When infection is mild, spots appear on the flowers in severe cases, the entire flower may be covered with spore masses and a blighting effect is produced. All infected flowers should be cut off and destroyed and the plants be kept in a place where humidity is low, temperature is high and there is good ventilation. For prevention of *Botrytis* it is important to keep the plants dry during cool whether. The best control may be achieved by careful spraying with fungicide and by eliminating of host plants near the orchid house. A black spot disease on blooms of *Vanda tricolor* caused by *Glomerella* sp is controlled by spraying with a solution of bioquin 700 (o-quinolinol benzoate) before the appearance of the symptoms (Ark and Snyder, 1951).

1.2.1.5. Root Rots: - The fungus *Fusarium* is responsible for this disease which causes the destruction of the root tissue. *Pellicularia filamentosa* is also said to cause root

rot. The disease kills the seedling and retards the growth of mature plants. Affected plants should be treated with zineb or tersan (Bose and Yadav, 1989).

1.2.1.6. Orchid wilt: - This disease is caused by *Sclerotium rolfsii* which affects mostly *Cymbidium* and *Paphiopedilum* plants. The leaf base initially becomes yellow later turning brown. Warm humid weather encourages spread of the disease. Affected leaves should be removed and plants repotted in fresh medium after treatment with protective fungicide like pentachloro-nitro-benzene. Treatment with zineb or natriphene is effective (Bose and Yadav, 1989).

1.2.1.7. Anthracnose Orchid Spot: - It is a major disease, often superficial, caused by a variety of fungi. Plants damaged or weakened by poor growing conditions are often attacked. Excessive fertilization with nitrogen also makes plants susceptible to infection. The disease is caused by *Colletotrichum gleosporiodes*, C. *cinctum* several species and hybrids in the genera *Aerides, Cymbidium, Dendrobium, Vanda* etc. are the common host of this disease. The basic symptoms of this disease are raised pustules on flower buds, petals and sepals. Target spot type lesion often developed at tip or on the middle area of the leaves. The spots may be circular, oval, sunken and reddish in colour. Eradication of disease parts and / or organs by burning has been suggested. Spraying with captan or benomyl proves useful in controlling the disease (Ciferri, 1926).

1.2.1.8. *Cymbidium* tip burn: - The disease is caused by *Botrytis* species; the pathogen penetrates the leaf tips damaged by exudation of salts and spreads rapidly into the healthy tissues of the plant. The infected leaf tips becomes spotted the spots coalesce to ultimately result in the death of entire tip. Dead tips usually get coated by mass of powdery spores. Removal of dead tips and spray with fungicides such as bavistin, captan or dithane was suggested by Vij and Kaur, 1985.

1.2.1.9. Rust: - In the United States and other parts of the world, *Cattleya* and other orchids are subjected to the attack of a rust fungus *Hemileia americana.* The disease is characterized by yellow pustules covered with fine powdery masses of spores (Ark 1959). Treatment with copper fungicide or terson has been suggested.

1.2.1.10. Collar rot: - It is caused by *Sclerotium rolfsii.* The stem and leaf bases of *Cymbidium, Paphiopedilum* loose chlorophyll. The infected parts usually harbour white mycelium and sclerotia. The sclerotia may survive in soil, potting mixes, and on benches and pots for many years. The disease is checked by destroying the infected plants, practice of strict sanitation, proper sterilization of potting medium and use of carboxin like fungicide is most effective (Ark, 1959).

1.2.1.11. Damping off: - It is caused by number of pathogens like *Phytophthora palmivora, P. omnivora, Pythium ultimum* etc. The pathogen attacks seedlings of many species and hybrids including *Cattleya, Vanda.* Watery spots appear in leaves, which turn yellow/ black and die, the roots often collapse and eventually the entire plant dies. Sanitation, controlled irrigation, sterile potting media and containers and clean benches are usually recommended to prevent damping off. Seedling should be drenched at seven-day intervals with 50 mg/l Dexon or 1 part copper sulphate in 100,000 part of water (Vij and Kaur, 1985).

1.2.1.12. Botrytis flower spotting: - This disease affects *Cymbidium*, *Cattleya*, *Dendrobium* and *Phalaenopsis* particularly, and generally the older flowers of other genera. Very small dark spots appear on the flowers. If conditions are very moist, then a grey mould growth will appear. The best control is prevention: Removal and eradication of infected materials reduce the source of inoculum, increase the air movement, reduce humidity and increase night time temperatures (Wright, 1994).

1.2.1.13. Leaf and Stem rot of *Cymbidium*: - A *Phytophthora* species was isolated from blackened leaves and stems of infected *Cymbidium* plants. Cultural characters did not fit descriptions of any known *Phytophthora* species. It was concluded that a new *Phytophthora* species, described here as *Phytophthora multivesiculata* is the causal agent (Ilieva *et al.*, 1998).

1.2.1.14. Leaf Bloch of *Cymbidium*: The pathogen that causes a leaf blotch and a rot of cymbidium orchids in New Zealand has been identified as *Phytophthora multivesiculata* using morphological characteristics and Internal Transcribed Spacer (ITS) fingerprinting. It is widespread in the North Island of the country (Hill, 2004).

1.2.2. BACTERIAL DISEASES: -

1.2.2.1. Bacterial Soft Rot: - It is a serious disease of *Cattleya* orchids and is caused by *Erwinia carotovora*. The disease starts at the upper end of the leaf as small, water soaked and somewhat darker than normal green spot. Pseudobulb of the infected plants turns soft and pulpy and become yellow in colour. A foul smelling liquid sometimes starts to ooze from the bulb. It is difficult to control the disease, but spraying with agrimycin has certain beneficial effect (Limber, and Friedman, 1943).

1.2.2.2. Bacterial Brown Rot: - This disease is serious on *Paphiopedilum*. It is caused by a rod shaped bacteria *Erwinia cypripedii* as described by Hori (1911). The organism attacks the plants through wounds and cracks. Yellowing and browning of leaves indicate the presence of the disease. The infection spreads into the crown and causes death of the plant. The disease could be suppressed by merging infected plants in 1:2000 solution of quinolinol benzoate or natriphene for 1 to 2.5 hours.

1.2.2.3. Bacterial Brown Spot: - This is a disease of both seedling and full-grown plants and caused by *Xanthomonas cattleyae*. In the beginning, there appear a small water soaked spot on any part of the leaves. The lesion enlarges and soon the leaf become soft and dies. The disease may progress into the growing point often killing the plant (Bose and Yadav, 1989).

1.2.2.4. Bacterial leaf rot of *Odontioda*: An unknown disease causing leaf rot on leaves of *Odontioda* sp., which is an intergeneric hybrid of *Odontoglossum*, occurred in Tochigi Prefecture, Japan, during the summer of 1995. One bacterial species was almost purely isolated from the infected leaves. The isolated strains were pathogenic to *Odontioda* cv. Baiser orchids in the dark with high temperature stress and high humidity. Based on the results of API 20E strips and other physiological and biochemical tests, the strain was identified as *Enterobacter cloacae*. This is the first report of an orchid disease caused by *E. cloacae* (Takahashi, *et al.*, 1997).

1.2.2.5. Bacteria Leaf spot: The disease caused by *Acidovorax avanae* subsp. *cattleyae* has been reported on three genera of orchids viz. *Dendrobium, Oncidium, Rhynchostylis*, usually start as small brown soft water soaked areas which quickly expand over the entire leaf. The covering infected area is easily damaged releasing a bacterial 'soup' which is spread to healthy leaves by splashing water (Duff and Daly, 2002).

1.2.3. VIRAL DISEASES: -

Orchids are affected by more virus disease problems than most crops (Zettler *et al.*, 1990), reducing their commercial values considerably. Viruses cause a number of orchid diseases which can be prevented but are difficult to control, impossible to eliminate or cure, and generally lead to loss of plant. Viral infections are rare in wild orchid. Jensen (1952a; 1952b) listed 32 virus diseases to occur in orchids but it does not mean that there were 32 different kinds of

viruses. Viruses can be transmitted by insects, mites, nematodes, fungi, and parasitic plants. Human handling may also transmit viral diseases. Viruses can also be transmitted through vegetative propagation, sap and contact between plants, pots and greenhouse benches. *Cymbidium mosaic virus* (CymMV), tobacco mosaic virus-o (TMV-o) and *Odontoglossum ringspot virus* (ORSV) are the most common among orchid viruses. Viral diseases are not always easy to recognize. Sherpa *et al.* (2003) reported *Cymbidium mosaic virus* on *Cymbidium* for the first time from India. In Thailand CymMV was detected in 17 genera in 93% nurseries, while ORSV was detected in 4 genera (*Arachnis, Cattleya, Oncidium* and *Vanda*) in 40% of nurseries tested (Tanaka *et al.*, 1997). Symptoms of viral diseases often take the form of chlorotic spots of mottled areas, or sometimes more prominent rings, streaks and necrotic areas. In situation where certain viruses commonly cause symptomless infections, the viruses may spread systematically through the entire plant. These diseases are incurable. Orchid growers are likely to spread viral diseases unwittingly in the normal cause of horticulture operations. The best means of controlling viral diseases are cleanliness, eradication of insect vector and infected plants, use to clean tools, removal of weeds and other sanitation procedures. A solution of 2% formalin and 2% NaOH in water is an excellent disinfectant for tools. Infected plants must be destroyed or isolated carefully to prevent the spread of the disease. Shoot tip culture is the only way to save infected plants. Unfortunately, however, even this procedure is not complete cure because shoot tips of some plants are difficult or impossible to culture or cannot be freed of viruses through tissue culture.

1.3. BIOLOGICAL CONTROL: A CONCEPT

Biological control was established by trial and error and then practiced in agriculture long before the term itself came into use (Baker and Cook, 1974). It is conceivable to believe that the control of plant diseases can be done with chemicals but alternate method of controlling plant diseases are needed as the use of chemicals and pesticides is disturbing the natural ecosystem with sometime disastrous consequences. Baker and Cook (1974) have discussed the 'how and why' of biological control. Biological control offers a powerful means to increase yield by destruction or suppression of pathogen inoculums, protect plant against infection or increase the ability of plant to resist pathogen. The era of modern biological control, involving the deliberate transfer and introduction of natural enemies of insect pests, was launched 100 years ago with the highly successful introduction of the vadalia beetle from Australia to California in 1888 to control the cottony cushion scale of citrus. In 1914, the German plant pathologist C. F. von Tubuef wrote a somewhat speculative article entitled "Biologische Bekampfung von Pilzkrankheiten der Pflanzen." This is apparently the first reference in the scientific literature to the term "Biologische Bekampfung" or "biological control" (Baker, 1987). With the increasing public awareness of the environmental implications of the use of large quantities of pesticides in agricultural practices, alternative strategies for the control of plant diseases are being sought (Weller 1988). Many of these strategies involve the use of antibiotic-producing fluorescent pseudomonads, and other bacteria, as effective biological control agents (BCAs) (O'Sullivan and O'Gara 1992). Several commercial biocontrol products are available which protect seedlings from fungal diseases, e.g. 'No Gall', an *Agrobacterium* strain antagonistic to crown gall disease of fruit, 'Dagger G', a *Pseudomonas fluorescens* strain active against damping-off of cotton, and 'GlioGard', based on a strain of *Gliocadium virens* (Cook 1993; Ryder 1994). Despite these apparent successes, the uses of BCAs are generally not as

reliable as their chemical counterparts (Weller 1988). This may be due to the inherent variability of applying a living organism to the environment. As environmental conditions fluctuate, so the colonization and *in planta* activity of inocula may vary (Weller and Thomashow 1994). Biological control of foliar pathogens with antagonistic fungi offers an ideal remedy to problems arising out of constant fungicide application (Mathivanan *et al.*, 2000). Govendasamy and Balasubramaniam (1989) have reported the biocontrol potential of *Trichoderma harzianum* in controlling rust disease in groundnut under green house condition. The potential of *Trichoderma* spp. in foot rot management in black pepper has been clearly established (Rajan *et al.*, 2002). The key to achieving successful, reproducible biological control is the gradual appreciation that knowledge of the ecological interactions taking place in phylosphere and rhizosphere is required to predict the conditions under which biocontrol can be achieved (Deacon, 1994; Whipps, 1997) and, indeed, may be part of the reason why more biocontrol agents are reaching the market-place (Fravel, 1999; Whipps and Lumsden, 2001; Whipps and Davies, 2000). This type of work requires a study not only of any potential biocontrol agent *per se* but also its interactions with the crop, the natural resident microbiota and the environment as well. Biocontrol involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment. Even in model laboratory systems, the study of biocontrol involves interactions among a minimum of three organisms. Therefore, despite its potential in agricultural applications, biocontrol is one of the most poorly understood areas of plant-microbe interactions. The complexity of these systems has influenced the acceptance of biocontrol as a means of controlling plant diseases in two ways. First, practical results with biocontrol have been variable. Thus, despite some stunning successes

with biocontrol agents in agriculture, there remains a general skepticism born of past failures (Weller, 1988). Second, progress in understanding an entire system has been slow. Recently, however, substantial progress has been made in a number of biocontrol systems through the application of genetic and mathematical approaches that accommodate the complexity. Biocontrol of soilborne diseases is particularly complex because these diseases occur in the dynamic environment at the interface of root and soil known as the rhizosphere, which is defined as the region surrounding a root that is affected by it. The rhizosphere is typified by rapid change, intense microbial activity, and high populations of bacteria compared with non-rhizosphere soil. Plants release metabolically active cells from their roots and deposit as much as 20% of the carbon allocated to roots in the rhizosphere, suggesting a highly evolved relationship between the plant and rhizosphere microorganisms. The rhizosphere is subject to dramatic changes on a short temporal scale rain events and daytime drought can result in fluctuations in salt concentration, pH, osmotic potential, water potential, and soil particle structure. The complexity of the root-soil interface must be accommodated in the study of biocontrol, which must involve whole organisms and ultimately entire communities, if we are to understand the essential interactions in soil in the field. The challenge in elucidating mechanisms of biocontrol is in reducing the complexity to address tractable scientific questions. One of the most effective approaches toward the identification of critical variables in a complex system has been genetics. The study of mutants can be conducted in simplified laboratory systems or in the field, thus making accessible the examination of particular genetic changes and the associated biochemical characteristics in the real world.

1.4. MECHANISMS OF BIOCONTROL

The mechanisms of biological control of plant pathogens by antagonistic bacteria and fungi have been a subject of many studies in the past two decades (Janisiewicz, *et al.,* 2000). Several mechanisms, operating alone or in concert, are known to be involved in antagonistic interactions in the phyllosphere and rhizosphere. Nutrient competition, antibiosis and mycoparasitism are the major mechanisms. Additional mechanisms, such as induced resistance, production of biosurfactants, interference with pathogen-related enzymes and undoubtedly a number of still unknown mechanisms may complete the microbial arsenal (Elad, 1996). In particular, mechanistic studies have benefited from molecular biology by identifying, deleting and supplementing genes responsible for mechanisms such as antibiotic production. Knowledge of mechanisms involved in biocontrol is important for estimating and predicting its reliability and selection of better strains. Besides other criteria, the choice of an antagonist with its characteristic mechanisms depends on the stage of the life cycle of the pathogen the antagonist is aimed at. Allowable interaction times and niche characteristics determine the suitability of certain mode of action during different developmental stages of the pathogen.

1.4.1. Mechanism of action of bacterial antagonist against bacterial pathogen

In the last few years there have been relatively few studies of bacteria applied to seeds and roots for the purpose of controlling bacterial diseases. One example, is the application of non-pathogenic strains of *Streptomyces* to control scab of potato (*Solanum tuberosum*) caused by *Streptomyces scabies* (Ryan and Kinkel, 1997; Neeno-Eckwall and Schottel, 1999). Here biocontrol may operate through antibiosis or competition for space or nutrients in the rhizosphere. Antagonism of *Pseudomonas* strains toward *Erwinia carotovora* can be attributed to production of the siderophore pseudobactin (Kloepper *et al.,* 1980). In contrast, *Pseudomonas*

fluorescens F113 was shown to control the soft rot potato pathogen *Erwinia carotovora* subsp *atroseptica* by production of the antibiotic 2, 4-diacetylphloroglucinol (DAPG). Some evidence was also obtained that siderophore production by *P. fluorescens* F113 may play a role in biocontrol of potato soft rot under iron-limiting conditions, but DAPG appears to be the major biocontrol determinant. *Pseudomonas* species may also control crown gall disease in many dicotyledonous plants caused by *Agrobacterium tumefaciens* (Khmel *et al.*, 1998).

1.4.2. Mechanism of action of bacterial antagonist against fungal pathogen

The study in this area continues to increase at a rapid rate, stimulated by the increasing ease with which molecular techniques can be applied to answer questions concerning distribution, and occurrence and relative importance of specific modes of action. Although a range of different bacterial genera and species has been studied, the overwhelming numbers of papers have involved the use of *Pseudomonas* species (Whipps, 2001). Clearly, *Pseudomonas* species must have activity but it begs the question as to the features that make this genus so effective and the choice of so many workers. Pseudomonads are characteristically fast growing, easy to culture and manipulate genetically in the laboratory, and are able to utilize a range of easily metabolizable organic compounds, making them amenable to experimentation. But, in addition, they are common rhizosphere organisms and must be adapted to life in the rhizosphere to a large extent (deWeger *et al.*, 1995; Marilley and Aragno, 1999). A few specific examples of the modes of action involved with bacterial biocontrol of fungal pathogens in the rhizosphere and spermosphere are given below.

1.4.2.1. *Antibiosis*

Biocontrol is often attributed to antibiosis. In many biocontrol systems that have been studied, one or more antibiotics have been shown to play a role in

disease suppression. The fact that antibiosis is a common mechanism of biocontrol may be due to a bias in choice of organisms for study. Alternatively, it may be due to the attractiveness of the antibiosis hypothesis, or antibiosis may be simply a highly effective mechanism for suppressing pathogens in the rhizosphere (Handelsman and Stabb 1996). There are numerous reports of the production of antifungal metabolites (excluding metal chelators and enzymes) produced by bacteria *in vitro* that may also have activity *in vivo*. These include ammonia, butyrolactones, 2,4-diacetylphloroglucinol (Phl), HCN, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (Pln), viscosinamide, xanthobaccin, and zwittermycin A as well as several other uncharacterized moieties (Milner *et al.*, 1996; Kang *et al.*, 1998; Kim *et al.*, 1999; Nakayama *et al.*, 1999). To demonstrate a role for antibiotics in biocontrol, mutants lacking production of antibiotics or over-producing mutants have been used (Bonsall *et al.*, 1997; Chin-A-Woeng *et al.*, 1998; Nowak-Thompson *et al.*, 1999). Alternatively, the use of reporter genes or probes to demonstrate production of antibiotics in the rhizosphere is becoming more commonplace (Kraus and Loper, 1995; Raaijmakers *et al.*, 1997; Chin-A-Woeng *et al.*, 1998). Significantly, both Phl and PCA have been isolated from the rhizosphere of wheat following introduction of biocontrol strains of *Pseudomonas* (Thomashow *et al.*, 1990; Bonsall *et al.*, 1997; Raaijmakers *et al.*, 1999), finally confirming that such antibiotics are produced *in vivo*. Further, Phl production in the rhizosphere of wheat was strongly related to the density of the bacterial population present and the ability to colonize roots (Raaijmakers *et al.*, 1999). PCA from *Pseudomonas aureofaciens* Tx-1 has even been used as a direct field treatment for the control of dollar spot (*Sclerotinia homeocarpa*) on creeping bentgrass (*Agrostis palustris*) (Powell *et al.*, 2000).

1.4.2.2. *Competition for iron*

Although competition between bacteria and fungal plant pathogens for space or nutrients has been known to exist as a biocontrol mechanism for many years (Whipps, 1997) the greatest interest recently has involved competition for iron. Under iron-limiting conditions, bacteria produce a range of iron chelating compounds or siderophores which have a very high affinity for ferric iron. These bacterial iron chelators are thought to sequester the limited supply of iron available in the rhizosphere making it unavailable to pathogenic fungi, thereby restricting their growth (O'Sullivan and O'Gara, 1992; Loper and Henkels, 1999). Studies have clearly shown that the iron nutrition of the plant influences the rhizosphere microbial community structure (Yang and Crowley, 2000). In general siderophores are grouped as hydroxamate, phenol /catecholates and carboxylate (Neilands, 1981). Ferric hydroxamate complex is more stable and predominant in rhizosphere (O'Sullivan and O'Gara, 1992) Iron competition in pseudomonads has been intensively studied and the role of the siderophore known as pyoverdine or pseudobactins that fluoresce under UV light (Buysens, *et al.*, 1996) produced by many *Pseudomonas* species has been clearly demonstrated in the control of *Pythium* and *Fusarium* species, either by comparing the effects of purified pyoverdine with synthetic iron chelators or through the use of pyoverdine minus mutants (Loper and Buyer, 1991; Duijff *et al.*, 1993). Pseudomonads also produce two other siderophores, pyochelin and its precursor salicylic acid, and pyochelin is thought to contribute to the protection of tomato plants from *Pythium* by *Pseudomonas aeruginosa* 7NSK2 (Buysens *et al.*, 1996). The dynamics of iron competition in the rhizosphere are often complex. For example, some siderophores can only be used by the bacteria that produce them (Ongena *et al.*, 1999), whereas others can be used by many different bacteria (Loper and Henkels, 1999). Different environmental factors can also influence the quantity of siderophores produced (Duffy and

Defago, 1999). There is also the further complication that pyoverdine and salicylate may act as elicitors for inducing systemic resistance against pathogens in some plants (Metraux *et al.*, 1990; Leeman *et al.*, 1996b).

1.4.2.3. *Parasitism and production of extracellular enzymes*

The ability of bacteria, especially actinomycetes, to parasitize and degrade spores of fungal plant pathogens is well established (El-Tarabily *et al.*, 1997). Assuming that nutrients pass from the plant pathogen to bacteria, and that fungal growth is inhibited, the spectrum of parasitism could range from simple attachment of cells to hyphae, as with the *Enterobacter cloacae–Pythium ultimum* interaction (Nelson *et al.*, 1986) to complete lysis and degradation of hyphae as found with the *Arthrobacter–Pythium debaryanum* interaction (Mitchell and Hurwitz, 1965). If fungal cells are lysed and cell walls are degraded then it is generally assumed that cell wall degrading enzymes produced by the bacteria are responsible, even though antibiotics may be produced at the same time. Considerable effort has gone into identifying cell wall degrading enzymes produced by biocontrol strains of bacteria even though relatively little direct evidence for their presence and activity in the rhizosphere has been obtained. For example, biocontrol of *Phytophthora cinnamomi* root rot of *Banksia grandis* was obtained using a cellulase-producing isolate of *Micromonospora carbonacea* (El-Tarabily *et al.*, 1996) and control of *Phytophthora fragariae* var. *rubi* causing raspberry root rot was suppressed by the application of actinomycete isolates that were selected for the production of ß-1,3-, ß-1,4- and ß-1,6-glucanases (Valois *et al.*, 1996). The gram negative rod *Stenotrophomonas maltophila* W81 is a sugar beet rhizosphere isolate capable of conferring protection against *Pytium ultimum*-mediated damping off (Dunne *et al.*, 1997, 1998). Mutagenesis of *S. maltophila* W81 with Tn*5*-746cd demonstrated that this ability is mediated by proteolytic enzyme production (Dunne *et al.*, 1997). Protease and chitinase

activity has also been reported among fluorescent pseudomonds (Nielsen *et al.*, 1998; Nielsen, *et al.*, 2002). Chitinolytic enzymes produced by both *Bacillus cereus* and *Pantoea* (*Enterobacter*) *agglomerans* also appear to be involved in biocontrol of *Rhizoctonia solani* (Chernin *et al.*, 1995; Pleban *et al.*, 1997). Tn5 mutants of *E. agglomerans* deficient in chitinolytic activity were unable to protect cotton (*Gossypium barbardense*) and expression of the *chiA* gene for endochitinase in *Escherichia coli* allowed the transformed strain to inhibit *R. solani* on cotton seedlings.

1.4.2.4. *Induced resistance*

The definition of induced resistance was suggested by Kloepper *et al.* (1992) covered both biotic and abiotic inducers. Perhaps the greatest growth area in biocontrol in the last few years has been concerned with induced resistance defined as 'the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (inducing agents)' (Kloepper *et al.*, 1992). This has come about through the synergistic interaction of microbiologists, plant pathologists and plant scientists armed with an appropriate battery of molecular tools. The effect had previously often been overlooked through inadequate techniques or controls as well as the biocontrol agent exhibiting other modes of action at the same time. Most work has focused on the systemic resistance induced by non-pathogenic rhizosphere-colonizing *Bacillus* and *Pseudomonas* species in systems where the inducing bacteria and the challenging pathogen remained spatially separated and no direct interaction between the bacteria and pathogen was possible (Sticher *et al.*, 1997; van Loon, 1997). Such split root or spatial root inoculation experiments were used to demonstrate the phenomenon in radish (*Raphanus sativus*) and *Arabidopsis* against *Fusarium oxysporum* (Leeman *et al.*, 1996*a*; van Wees *et al.*, 1997) and in cucumber (*Cucumis sativus*) against *Pythium aphanidermatum* (Chen *et al.*, 1998). Various combinations of timing and position have indicated that induced

resistance also occurs in carnation (*Dianthus caryophyllus*) (van Peer *et al.*, 1991), tobacco (*Nicotiana tabacum*) (Maurhaufer *et al.*, 1994) and tomato (*Lycopersicon esculentum*) (Duijff *et al.*, 1997). Bacteria differ in ability to induce resistance, with some being active on some plant species and not others; variation in inducibility also exists within plant species (van Loon, 1997). The full range of inducing moieties produced by bacteria is probably not yet known, but lipopolysaccharides (Leeman *et al.*, 1995) and siderophores (Metraux *et al.*, 1990; Leeman *et al.*, 1996b) are clearly indicated. Although the phenotypic effects of root inoculation with bacteria may be similar to treatment with abiotic agents or microorganisms that cause localized damage, the biochemical and mechanistic changes appear to be subtly different. This has resulted in the term induced systemic resistance (ISR) for bacterially-induced resistance and systemic acquired resistance for the other forms (Pieterse *et al.*, 1996). The major differences are that pathogenesis-related (PR) proteins such as chitinases, ß-1,3-glucanases, proteinase inhibitors and one or two other rarer types, are not universally associated with bacterially induced resistance (Hoffland *et al.*, 1995) and salicylic acid (a known inducer of SAR) is not always involved in expression of ISR, but this is dependent on bacterial strain and host plant involved (Pieterse *et al.*, 1996; de Meyer *et al.*, 1999;). Ethylene responsiveness may also be required at the site of inoculation of the inducing bacteria for ISR to occur (Knoester *et al.*, 1999).

1.4.2.5. *Plant growth-promoting rhizobacteria (PGPR)*

In recent years, considerable attention has been paid to Plant Growth Promoting Rhizobacteria (PGPR) as the best choice in place of agrochemicals, to facilitate the biocontrol of soil and seed borne plant pathogens (Weller, 1988; Haas and Defago, 2005). Most of the bacteria exhibiting Plant Growth Promotory (PGP) activity belong to Gram-negative group and among these

fluorescent pseudomonads are the most widely studied (Duffy and Defago, 1999; Siddiqui and Shaukat, 2003). PGPR increase plant growth indirectly either by the suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens (microorganisms which reduce plant growth but without obvious symptoms). Alternatively, PGPR may increase plant growth in other ways, for example, by associative N_2 fixation (Hong *et al.*, 1991), solubilizing nutrients such as phosphorus (Whitelaw, 2000), promoting mycorrhizal function (Garbaye, 1994), regulating ethylene production in roots (Glick, 1995), releasing phytohormones (Arshad and Frankenberger, 1991; Beyeler *et al.*, 1999), and decreasing heavy metal toxicity (Burd *et al.*, 1998). It has been suggested that the two groups should be reclassified into biocontrol plant growth-promoting bacteria (biocontrol PGPB) and PGPB (Bashan and Holguin, 1998). To date this proposal does not seem to have been widely accepted, but it does highlight the need to consider the full ecological interactions taking place following application of bacteria to seeds and roots that lead to plant growth promotion. It is also important to remember that deleterious rhizobacteria that inhibit plant growth are also known (Nehl *et al.*, 1996) which can influence such interactions.

Irrespective of mode of action, a key feature of all PGPR is that they all colonize roots to some extent. In some cases this may involve specific attachment through, pili, as with the attachment of *Pseudomonas fluorescens* 2-79 to the surface of wheat roots (Vesper, 1987). Differences in colonization between a fluorescent pseudomonad and isogenic flagella mutants prompted the conclusion that flagella are required for colonization of potato roots (de Weger *et al.*, 1995). When colonizing a root environment, an organism is confronted with a complex array of parameters such as water content, temperature, pH, soil types, composition of root exudates, mineral content and other microorganisms. Numerous studies have been conducted to assess

the contribution of each of these parameters. For example, the colonization of a fluorescent *Pseudomonas* strain in the potato rhizosphere was reported to be 10-fold greater in a sandy loam soil than in a clay loam soil (Bahme and Schroth, 1987) and another strain performed better in a sandy soil than in a peat soil (Kloepper *et al.*, 1980). Although these experiments suggest that soil texture may have a direct influence on the colonization of these strains, other indirect factors associated with these soil types could also provide underlying reasons for the differences in colonization. Colonization may involve simply root surface development but, endophytic colonization of the root is also known, and the degree of endophytic colonization depends on bacterial strain and plant type. Endophytic growth in roots has been recorded with the PGPR *Bacillus polymyxa* Pw-ZR and *Pseudomonas fluorescens* Sm3-RN on spruce (*Picea glauca x P. engelmannii*) (Shishido *et al.*, 1999), with the biocontrol strains of *Bacillus* sp. L324-92R_{12} and *P. fluorescens* 2-79RN_{10} on wheat (Kim *et al.*, 1997) and with several that induce resistance such as *Bacillus pumilus* SE34 and *P. fluorescens* 63-28 on pea (*Pisum sativum*) (Benhamou *et al.*, 1996*a*, *b*; M'Piga *et al.*, 1997), *P. fluorescens* CHA0 on tobacco (Troxler *et al.*, 1997) and *P. fluorescens* WCS417r on tomato (Duijff *et al.*, 1997). These endophytic bacteria may be in a particularly advantageous ecological position in that they may be able to grow and compete on the root surface, but also may be capable of developing within the root, relatively protected from the competitive and high-stress environment of the soil. Indeed, many seeds, roots and tubers are normally colonized by endophytic bacteria (McInroy and Kloepper, 1995; Sturz *et al.*, 1999). Any plant resistance encountered must be minimal, although, in many cases, sufficient to allow ISR to develop. The localized signaling between plant and bacteria within the root environment deserves further study. Certainly, use of mutants and promoter probe techniques are beginning to identify genes in bacteria that are important in colonization and these are often related to

nutrient uptake (Bayliss *et al.*, 1997; Roberts *et al.*, 2000). Such nutrient uptake genes may also play a role in biocontrol by aiding the uptake and metabolism of nutrients that stimulate germination of pathogen propagules (Maloney *et al.*, 1994).

The ability to colonize seeds is also an important feature for many bacterial biocontrol agents. *Pseudomonas chlororaphis* MA342 is applied to cereal seeds to control many seed and soil-borne pathogens and has been found to colonize specific areas of the seed coat (Tombolini *et al.*, 1999). After inoculation, the bacteria were found under the seed glume (or husk), but after planting they were found to colonize the glume cells epiphytically. At present, a good colonizing *Pseudomonas* strain is determined by testing its performance *in vivo*. As more knowledge on the traits needed for efficient colonization becomes available, it may be possible to select strains with defined characteristics.

1.4.3. Mechanism of action of fungal antagonist against bacterial pathogen

In the last few years there have been no clear examples of fungi used to control bacterial plant pathogens in the rhizosphere or spermosphere. The reasons for this are unclear but could perhaps indicate an area that deserves further research in the future (Whipps, 2001).

1.4.4. Mechanism of action of fungal antagonist against fungal pathogen

There are a variety of fungal species and isolates that have been examined as biocontrol agents but *Trichoderma* species clearly dominate, perhaps reflecting their ease of growth and wide host range (Whipps and Lumsden, 2001). There has been an upsurge in interest in non-pathogenic *Pythium* species, particularly *P. oligandrum* where additional modes of action have been determined recently, and a continued interest in well-established saprotrophic antagonists such as non-pathogenic *Fusarium* species, non-pathogenic binucleate *Rhizoctonia* isolates and *Phialophora* species, as well as mutualistic symbionts including mycorrhizal fungi

such as *Glomus intraradices*. The most common pathogen targets are *Pythium* species, *Fusarium* species and *Rhizoctonia solani* reflecting their world-wide importance and perhaps their relative ease of control under protected cropping systems, although numerous other pathogens have been examined. Some specific examples of the modes of action found to occur in the rhizosphere and spermosphere during interactions between fungi and fungal plant pathogens are given below.

1.4.4.1. *Competition*

There have been relatively few studies on competition for nutrients, space or infection sites between fungi in the rhizosphere and spermosphere recently. Competition for carbon, nitrogen and iron has been shown to be a mechanism associated with biocontrol or suppression of *Fusarium* wilt in several systems by non-pathogenic *Fusarium* and *Trichoderma* species (Mandeel and Baker, 1991; Couteadier, 1992; Sivan and Chet, 1989) and competition for thiamine as a significant process in the control of *Gaeumannomyces graminis* var. *tritici* by a sterile red fungus in the rhizosphere of wheat (Shankar *et al.*, 1994). Many studies have shown a relationship between increased colonization of the rhizosphere by a non-pathogen, associated subsequently, with disease suppression. This is well established for non-pathogenic strains of *Fusarium oxysporum* controlling pathogenic *F. oxysporum* on a variety of crop plants (Eparvier and Alabouvette, 1994; Postma and Rattink, 1991), hypovirulent or non-pathogenic binucleate strains of *Rhizoctonia* species to control pathogenic isolates of *R. solani* (Herr, 1995) and several fungi including *Phialophora* species, *Gaeumannomyces graminis* var. *graminis* and *Idriella bolleyi* as well as several non-sporulating fungi, to control *G. graminis* var. *tritici* (Deacon, 1974; Wong and Southwell, 1980; Kirk and Deacon, 1987; Shivanna *et al.*, 1996). As just one example, *I. bolleyi* exploits the naturally senescing cortical cells of cereal roots

during the early stages of the crop and out competes *G. graminis* var. *tritici* for infection sites and nutrients. Rapid production of spores, which are then carried down the root by water, continue the root colonization process and this is suggested to be a key feature in the establishment of the biocontrol agent on the root (Lascaris and Deacon, 1994; Allan *et al.*, 1992; Douglas and Deacon, 1994). Mycorrhizal fungi are also strong candidates for providing biocontrol through competition for space by virtue of their ecologically obligate association with roots. Ectomycorrhizal fungi because of their physical sheathing morphology may well occupy normal pathogen infection sites.

1.4.4.2. *Antibiosis*

Although production of antibiotics by fungi involved in biocontrol is a well-documented phenomenon (Howell, 1998; Sivasithamparam and Ghisalberti, 1998), there is little recent work clearly demonstrating production of antibiotics by fungi in the rhizosphere and spermosphere. Unlike the situation with biocontrol bacteria, there appear to be no detailed studies in biocontrol fungi of genes coding for antibiotic synthesis. Mutants with raised or decreased production of antibiotics are either natural spontaneous ones or generated by UV or chemical mutagenesis, with inherent problems of pleiotropic gene effects, rather than targeted gene disruption (Howell and Stipanovic, 1995; Graeme-Cook and Faull, 1991; Wilhite *et al.*, 1994; Fravel and Roberts, 1991). Consequently, clear identification and understanding of the role of antibiotics in disease control lags far behind that in bacteria and needs to be addressed. Antibiotic production by fungi exhibiting biocontrol activity has most commonly been reported for isolates of *Trichoderma / Gliocladium* (Howell, 1998) and *Talaromyces flavus* (Kim *et al.*, 1990; Fravel and Roberts, 1991) although in the last few years antibiotics have been at least partially characterized in *Chaetomium globosum* (Di Pietro *et al.*, 1992). *Minimedusa*

polyspora (Beale and Pitt, 1995) and *Verticillium biguttatum* (Morris *et al.*, 1995). Of particular interest are those studies where antibiotic production has a definite link to biocontrol. For example, *Trichoderma* (*Gliocladium*) *virens* comprises P and Q group strains, based on their antibiotic profiles (Howell, 1999). Strains of P group produce the antibiotic gliovirin which is active against *Pythium ultimum* but not against *Rhizoctonia solani* AG-4. Strains of the Q group produce the antibiotic gliotoxin which is very active against *R. solani* but less so against *P. ultimum*. Gliotoxin production by *Trichoderma* is also thought to be responsible for cytoplasmic leakage from *R. solani* observed directly on membranes in potting mix (Harris and Lumsden, 1997).

1.4.4.3. *Induced resistance*

As with bacteria, the ability of fungi to induce resistance in plants and provide biocontrol has gradually been receiving more attention in the last few years. A considerable number of fungi previously described to provide biocontrol by mechanisms such as competition, antibiosis, mycoparasitism or direct growth promotion are now thought to provide control, at least in part, by this mechanism. These include saprotrophs such as non-pathogenic *Fusarium* isolates (Hervas *et al.*, 1995; Larkin *et al.*, 1996; Postma and Luttikholt, 1996; Fuchs *et al.*, 1997, 1999; Duijff *et al.*, 1998; Larkin and Fravel, 1999), *Trichoderma* species (Yedidia *et al.*, 1999), *Pythium oligandrum* (Benhamou *et al.*, 1997; Rey *et al.*, 1998), non-pathogenic binucleate *Rhizoctonia* isolates (Poromarto *et al.*, 1998; Xue *et al.*, 1998; Jabaji-Hare *et al.*, 1999), and *Penicillium oxalicum* (de Cal *et al.*, 1997) as well as mutualistic biotrophs such as mycorrhizal fungi (Volpin *et al.*, 1995; Dugassa *et al.*, 1996; Morandi, 1996; St Arnaud *et al.*, 1997). However, not all these studies used the strict criterion of spatial separation between application of the biocontrol fungus and the challenging pathogen to define induced resistance. Indeed, some simply measured changes

in enzymes, PR-proteins or cell wall characteristics found to be induced in plants through SAR without involvement of a pathogen at all (Volpin *et al.*, 1995; Morandi, 1996; Yedidia *et al.*, 1999; Rey *et al.*, 1998). However, spatial or temporal separation experiments have indicated that increased levels of chitinases, ß-1,3 glucanases, ß-1,4 glucosidase, PR-1 protein, and peroxidase as well as cell wall appositions and phenolics may be associated with induced resistance due to fungi (Benhamou *et al.*, 1997; Fuchs *et al.*, 1997; Duijff *et al.*, 1998; Xue *et al.*, 1998; Jabaji-Hare *et al.*, 1999). The elicitors responsible for inducing resistance are not known in detail. *Trichoderma* species produce a 22 kDa xylanase that, when injected in plant tissues, will induce plant defense responses including K^+, H^+ and Ca^{2+} channeling, PR protein synthesis, ethylene biosynthesis, and glycosylation and fatty acylation of phytosterols (Bailey and Lumsden, 1998). However, whether such a system is active in roots exposed to *Trichoderma* is not known. Pectic oligogalacturonides released after hydrolysis by a non-pathogenic binucleate *Rhizoctonia* isolate may act as elicitors of defense responses in bean (*Phaseolus vulgaris*) (Jabaji-Hare *et al.*, 1999).

1.4.4.4. *Mycoparasitism*

There is a huge literature on the ability of fungi to parasitize spores, sclerotia, hyphae, and other fungal structures and many of these observations are linked with biocontrol (Jeffries and Young, 1994; van den Boogert and Deacon, 1994; Madsen and de Neergaard, 1999; Mischke, 1998; Al-Rawahi and Hancock, 1998; Davanlou *et al.*, 1999). However, most of the microscopical observations concerning mycoparasitism have come from *in vitro* studies or sterile systems (Benhamou and Chet, 1997; Inbar *et al.*, 1996; Cartwright *et al.*, 1997; Benhamou *et al.*, 1999; Davanlou *et al.*, 1999) and examples clearly demonstrating mycoparasitism in the rhizosphere or spermosphere are rare (Lo *et al.*, 1998). However, indirect population dynamic studies showed that

mycelium of *Rhizoctonia solani* in the rhizosphere of potato was a prerequisite for development of the mycoparasite *Verticillium biguttatum* (van den Boogert and Velvis, 1992) and rhizosphere competence was strongly related to biocontrol in mycoparasite isolates of *Trichoderma* species (Sivan and Harman, 1991; Peterbauer *et al.*, 1996; Thrane *et al.*, 1997; Harman and Björkman, 1998).

The process involved in mycoparasitism may consist of sensing the host, followed by directed growth, contact, recognition, attachment, penetration, and exit. Although not all these features occur in every fungal–fungal interaction, the key factor is nutrient transfer from host to mycoparasite. Directed growth of hyphae of *Trichoderma* to hyphae of *Rhizoctonia solani* prior to penetration has often been observed (Chet *et al.*, 1981). However, the factors involved in controlling directed growth in these systems have not been fully characterized. Similarly, the factors controlling recognition and binding between fungal host and parasite are not yet clear. This process may involve hydrophobic interactions or interactions between complementary molecules present on the surface of both the host and the mycoparasite such as between lectins and carbohydrates. With *Trichoderma*, there is good evidence of lectin production by both parasite and host *Corticum* (*Sclerotium*) *rolfsii* and involvement of lectins in the differentiation of mycoparasitism-related structures (Inbar and Chet, 1994; Neethling and Nevalainen, 1996). As penetration or cell wall degradation are frequently observed during mycoparasitism, great emphasis has been placed on characterizing and cloning the extracellular enzymes such as ß-1,3 glucanases, chitinases, cellulases, and proteases produced by fungal biocontrol strains (Haran *et al.*, 1996; Peterbauer *et al.*, 1996; Archambault *et al.*, 1998; Deane *et al.*, 1998). By manipulating their activity through construction of 'overproducing' mutants, enzyme-negative mutants or even transgenic plants expressing the enzyme, a role for their production in biocontrol has been implied. Several fungi have been

examined in this way including *Talaromyces flavus* (Madi *et al.*, 1997), but this type of work has essentially focused on *Trichoderma* species. Transformants of *T. harzianum*, overproducing proteinase encoded by *prb1*, provided up to a 5-fold increase in control of damping-off in cotton caused by *Rhizoctonia solani* (Flores *et al.*, 1996). Interestingly, the best protection was provided by a strain which produced only an intermediate level of proteinase activity and it was suggested that very high levels of proteinase production might cause degradation of other enzymes which are important in the mycoparasitic process (Flores *et al.*, 1996). In this regard, chitinases have received the greatest attention in mycoparasitism. The combination of chitinases as well as other cell wall-degrading enzymes differs between species and strains (Lorito, 1998) and chitinases are differently expressed during mycoparasitism (Mach *et al.*, 1999; Zeilinger *et al.*, 1999). For example, an *N*-acetyl hexosaminidase (CHIT 102) was the first to be induced in *T. harzianum* T-Y, but as early as 12 h after contact with its host *Sclerotium rolfsii*, its activity diminished, while that of another *N*-acetylhexosaminidase increased (Haran *et al.* 1996).

The final evidence for a role for cell wall-degrading enzymes in biocontrol involves the expression of fungal genes in transgenic plants. For example, an endochitinase from *Trichoderma harzianum* has been transformed into tobacco and potato and the transgenic plants showed a high level of resistance to a broad spectrum of diseases (Lorito, 1998). Similarly, transgenic apple trees expressing an endochitinase from *T. harzianum* also exhibited increased resistance to apple scab caused by *Venturia inaequalis* although plant growth was reduced (Bolar *et al.*, 2000). The potential consequently exists to combine different enzymes in transgenic plants to obtain synergistic biocontrol and these experiments are underway (Lorito, 1998; Bolar *et al.*, 2000).

1.4.4.5. *Plant growth promotion and rhizosphere competence*

The terminology associated with biocontrol in the rhizosphere and with soil–plant–microbe interactions has gradually become more complex through the use of a range of descriptive rather than mechanistic terms such as plant growth promotion and rhizosphere competence. Much like the situation with PGPR, many saprotrophic fungi, particularly certain isolates of *Trichoderma* species, can provide plant growth promotion in the absence of any major pathogens (Whipps, 1997; Inbar *et al.*, 1994). In many cases these studies are restricted to simple observations of improved plant growth with no indication of the possible mechanisms involved, although there are exceptions. For example, *Trichoderma harzianum* 1295–27 was shown to solubilize phosphate and micronutrients that could be made available to provide plant growth (Altomare *et al.*, 1999). This situation is compounded by the fact that many proven fungal biocontrol agents including some *Trichoderma* species, binucleate *Rhizoctonia* isolates and *Pythium oligandrum* can provide improved plant growth in the absence of pathogens (Windham *et al.*, 1986; Shivanna *et al.*, 1996; Wulff *et al.*, 1998; Harris, 1999). Further, colonization of the surface of the seeds or roots or behaviour as endophytes has frequently been seen to be a desirable trait for biocontrol activity (Kleifeld and Chet, 1992; Harman and Bjorkman, 1998) and although there is a clear relationship between rhizosphere colonization and biocontrol activity with some isolates of biocontrol fungi such as *Trichoderma* species, non-pathogenic *Fusarium* species, *P. oligandrum*, *Verticillium biguttatum*, and *Talaromyces flavus* (Ahmad and Baker, 1988; Couteadier *et al.*, 1993; van den Boogert and Velvis, 1992; Al-Rawahi and Hancock, 1997; Lo *et al.*, 1996; Tjamos and Fravel, 1997; Nagtzaam and Bollen, 1997; Bjorkman *et al.*, 1998), this is not always the case. Indeed transient plant growth inhibition following application of some biocontrol agents to seeds or roots is known (Wulff *et al.*, 1998; Bailey and Lumsden, 1998). Consequently, it is important to appreciate

that just because a microorganism can grow in the rhizosphere or spermosphere; it may not automatically provide biocontrol or plant growth promotion. Similarly, the converse is true. A proven biocontrol agent of a soilborne plant pathogen may not always be capable of colonizing the rhizosphere or providing plant growth promotion.

1.5. THESIS OBJECTIVE

Literature survey of the introductory chapter leads to formulate the objective stated below:

- Field survey, symtomological and histopathological study, isolation of pathogen(s) from infected region of the plant parts and pathogenicity test to confirm pathogens.
- Isolation and characterization of biocontrol agents from rhizospheric soil of healthy plant as well as from soils of different places of Darjeeling and its adjoining area.
- Study on biocontrol potentiality of the isolated organisms against the causal organism(s) of *Cymbidium* rot and finally screening out the potential biocontrol organism which will be used for further study.
- Study of the mechanism of action of the biocontrol agents.
- Optimization of different physiological factors on growth of the biocontrol agent for large-scale biomass production; powder formulation of the biocontrol agent and test for survivality.
- *In vivo* evaluation on the potentiality of the biocontrol agent.

These objectives are pursued via accumulation of literature on the subject and the aim of the following chapters of this thesis is to satisfy the said objectives.

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