Chapter-II

REVIEW OF LITERATURE
REVIEW OF LITERATURE

There are several varieties of these leafy vegetables either in the wild or under cultivation in the rural areas. Vegetables constitute the most important and inexpensive component of a balanced diet, which people now realize due to their high nutritive values indispensable for the body. During the last two decades considerable emphasis has been laid on increasing production of vegetable crops in India. The age of civilization which influenced the drastic migration to urban centre’s has however had a great influence on the choice of vegetables used as food. Greens make up significant source of vitamins A, C, B, E and K. They are rich sources of minerals such as calcium, magnesium, iron and potassium. They are rich in fiber, extremely low in fat and carbohydrates and provide an excellent source of protein. In this investigation some important leafy vegetable crops viz. chuka (*Rumex acetosa* L.), spinach (*Spinacea oleracea* L.), fenugreek (*Trigonella foneum-graecum* L.), sepu (*Peucedonum graveolens* Benth and Hook.) and colocasia (*Colocasia esculanta* L.) they have been selected and attacked by many fungal diseases like, leaf spot, wilt, damping off, root rot, powdery mildew, leaf blight and dry rot diseases.

ISOLATION

*Alternaria species*

*Alternaria tenuissima* is a cosmopolitan fungus already identified in India on several hosts e.g. *Colocasia esculenta* (Solankure and Rao, 1972) *Cajanus cajan* (Kannaiyan and Nene, 1977) and *Ipomoea carnea* (Reddy and Rao, 1975). Whereas first report of *Alternaria tenuissima* causing leaf spot and fruit rot on *Solanum melongena* was reported by Raja et al. (2005) in India. There are reports of *Alternaria tenuissima* causing disease on blueberry and pepper in China, but there is no previous report of the pathogen on sorrel plants (Luan et al., 2007; Li et al., 2011) After two-three
days of inoculation, ash green colonies with whitish peripheral concentric rings are formed. Isolated fungus was identified as *Alternaria tenuissima* (Fries) Wiltshire (Simmons, 2007; Subramaniam, 1971).

The first report of *Alternaria alternata* causing leaf spot disease of aloe reported in India (Kamalakannan et al., 2008). *Alternaria alternata* has previously been reported as a leaf spot pathogen of tomato (Akhtar et al., 2004), stevia (Maiti et al., 2006) and pomegranate (Madhukar and Reddy, 1976). Similarly leaf spot (*Alternaria tenuissima*) occurs primarily in the spring during prolonged periods of cool wet weather, when spores are produced in abundance. In most cases only lower leaves are affected; however, instances do occur when severe infection completely defoliates the plant. Leaf symptoms appear as circular to irregular-shaped brown to grey lesions surrounded by a red border. Based on the symptoms, mycelial and conidial characters, the fungus was identified as *Alternaria alternata* (Ellis, 1971).

First report of *Alternaria alternata* causing leaf spot on *Aloe barbadensis* in India was reported by Lakshmi and Valluvarparidasan (2008). *Alternaria* late blight, caused by *Alternaria* sp. that is *Alternaria alternata, Alternaria tenuissima* and *Alternaria arborescens* species groups is one of the most common fungal diseases of pistachio. The disease can cause severe premature defoliation, staining of nutshells and moulding of the kernels, which reduce fruit quality. On foliage, the disease is characterized by the development of large necrotic lesions that eventually coalesce and consume the entire leaf (Pryor and Michailides, 2002).

Moldy heart disease caused by *Alternaria* sp. is frequently observed in red delicious apples in Argentina (Robiglio and López, 1995). There are previous reports of *Alternaria* species *Alternaria alternata* and *Alternaria chlamydospora* associated with discolouration of amaranth seeds (Noelting
et al., 2009a&b) but this is the first documented report of *Alternaria infectoria* affecting panicles and seeds of amaranth in Argentina. *Alternaria infectoria* has been reported on wheat in Argentina (Perelló et al., 2007). First report of *Alternaria infectoria* on amaranth (*Amaranthus caudatus* sp. *mantegazzianus*) in Argentina (Noelting, 2012). First report of *Alternaria infectoria* on leaf spot of gerbera (Mirkova and Konstantinova, 2003).

Losses caused due to post-harvest diseases are greater than generally realized because the value of fresh fruits and vegetables increases several-fold while passing from the field to the consumer (Eckert and Sommer, 1967). Species of *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*, and *Geotrichum* as well as to *Botrytis* have been reported as common post-harvest fungi (Splittstoesser, 1987; Adaskaveg et al., 2002). Leaf spot of aspm, *Alternaria* species are fungi widely distributed in the soil as normal components of its microbiota and are both saprophytes and plant pathogens. They are widespread in both humid and semi-arid regions and can infect growing plants in the field. *Alternaria* species cause plant diseases on many crops, affecting the leaves, stems, flowers and fruits. Total losses caused by this genus rank among the highest caused by any plant pathogen (Agrios, 2005). During storage, *Alternaria* may also spread from affected plant products to adjacent healthy ones by secondary infections (Barkai-Golan, 2008).

The first time reported a fungal leaf spot of *Chrysanthemum maximum* L. caused by *Alternaria chrysanthemi* Simmons and Crosier from Austrian Tyrol. He noticed that the disease on all green parts of the plants. Initially, round, pale grey spots appeared which enlarged rapidly to their final diameter of one cm when fully developed; they became grey to brownish black, often with a pale fleck at the centre and more or less distinct light and dark zonations (Schmidt, 1958). Whereas spots were
sufficiently numerous entire leaves withered reported *Alternaria chrysanthemi* from Florida. The first time reported *Alternaria* blight of *C. cinerariefalium* (Trev.) var. *pyrethrum* caused by *Alternaria tenuissima* from Bangalore.

The leaf blight of *Chrysanthemum morifolium* caused by *Alternaria tenuissima* (Fries) Dharwad in Karnataka. These spots circular in the beginning enlarge later and became irregular and turned to blackish brown or dark brown color. Eventually such spots covered entire leaf and coalescing of spots caused leaf blight, such blighted leaves were found to defoliate. On upper leaves also similar symptoms appeared flowers remained free from infection (Hegde, 1988).

While widespread occurrence of *Alternaria* leaf blight on sunflower from North Karnataka reported (Hiremath et al., 1990). Results indicated that there was higher incidence of *Alternaria* leaf blight compared to *Colletotrichum* leaf spot on turmeric crop in all the districts surveyed (Gorawar, 2004).

The *Alternaria* leaf blight of sunflower has been reported from India and elsewhere (Acimovic, 1969; Shane et al., 1981; Herr and Lipps, 1981; Kolte, 1984). The *Alternaria* leaf blight of sunflower caused by *Alternaria helianthi* (Hansf) to infect all aerial parts of plant viz. leaf, petiole, stem, floral parts and seeds. Initially, the disease appears in the form of small, scattered brown spots on the leaf lamina (Rao, 1965).

First Report of *Alternaria* species groups involved in disease complexes of hazelnut and walnut fruit (Belisario, 2004). Severe infection of *Alternaria* blight on leaf, stem, petiole and inflorescence including petals was described by many workers (Balasubramanyam and Kolte, 1980a&amp;b; Nargund and Nazeer, 1994).
First report in Israel of Aralia leaf spot caused by *Alternaria panax* (Levy et al., 2006). The symptoms on stems, leaves, flowers and seeds. On the leaves, spots were round at first pale grey and up to one cm diameter, later grey or brownish black, often with a whitish spot in the centre surrounded by pale and dark concentric rings. *Alternaria alternata* and *Fusarium oxysporum* are reported as important diseases of commercial chrysanthemums (Ellis, 1998).

Morphology of the pathogen the genus *Alternaria* was first described by Nees in 1817 with *Alternaria tenuis* as the type species. The conidial characteristics of the genus are uniform, attenuated and catenulate. Fries (1832) had proposed the *Alternaria alternata* as *Torula alternata* Pers. The reasons why the specific epithet ‘*alternata*’ should be used instead of the more commonly accepted one ‘*tenuis*’ was clearly stated by (Simmon, 1965). Keissler (1912) and Srinath and Sarwar (1965) had given the morphology of *Alternaria alternata* and *Alternaria tenuissima*.

**Fusarium species**

Mc Millan (1986) was reported variations among *Fusarium* isolates from cucumber. Taubenhaus (1920) and Owen (1956) concluded that the watermelon wilt *Fusarium* was pathogenic only to watermelon, not cucumber, muskmelon, pumpkin or squash. Owen (1955) found through cross inoculation studies with *F. oxysporum f. sp. cucumerinum* and *F. oxysporum f. sp. niveum* isolated from cucumber and watermelon in Florida, that *Fusarium* isolates were specifically pathogenic. A change from one forma specialis (*F.oxysporum f. sp. niveum*) to another forma specialis (*F. oxysporum f. sp. melonis*) has been reported (Bouhot, 1981) and one isolate of *F. oxysporum f. sp. cucumerinum* from the Netherlands is pathogenic to cucumber, muskmelon and watermelon (Geriagh and Blok, 1988). Similar concepts were established in *F. oxysporum f. sp.*
Review of Literature

*conglutinans* (Wollenweber Snyder and Hansen) which contains isolates attacking cruciferous plants (Armstrong and Armstrong, 1981). In term of pathogenicity *F. oxysporum f. sp. meloniss* on cucumber, results are not in line with the other studies (Armstrong and Armstrong, 1978).

The *Allium* sp. causal fungus was isolates were identified cultural and morphological characteristics as *Fusarium proliferatum* (Nelson et al., 1983; Nirenberg and O’Donnell, 1998). Previously, *Fusarium proliferatum* has been reported on onion in the north western USA (Mohan et al., 1997; Seefelder et al., 2002). *Fusarium proliferatum* and *Fusarium fumonisins* in garlic bulbs in Germany. *Fusarium proliferatum* was previously known to be associated with several diseases of plants, viz. damping-off of onion, foot-rot of corn (Farr et al., 1989). *Fusarium* head blight (FHB) in North America is primarily caused by *F. graminearum* Schwabe (teleomorph Gibbrella zeae (schwein) petch) (Bai et al., 2001; Parry et al., 1995; Schroeder and Christensen, 1963).

*Fusarium* species play a significant role as plant pathogens, causing a broad range of diseases on various host plants, such as vascular wilt, pre and post-appearance blight as well as root and stem rots (Pascale et al., 2002; Schollenberger et al., 2006). *Fusarium* is also considered as a vital genes associated with cereal mycology where this pre-harvest fungal infection prolongs until post-harvest and storage stage (Hussein et al., 1991; Mubatanhema et al., 1999; Ezekiel et al., 2008).

A number of morphologically related *Fusarium* species, namely: *Fusarium moniliforme*, *Fusarium proliferatum*, *Fusarium napiforme*, *Fusarium anthophilum*, *Fusarium dlamini*, *Fusarium thapsinum* and *Fusarium globosum* are occurring world-wide and can produce a group of structurally-related mycotoxins such as fumonisins (Pieckova and Jesenska, 2001; Visconti et al., 1999).
The ability of *Fusarium proliferatum* (9 isolates) to produce fumonisins was tested by (Castella et al., 1999) and shown that all of the isolates were fumonisin producers. According to (Vesonder, 1986) each of the 12 strains of *Fusarium proliferatum* produced moniliformin (45–16,000 ppm), fumonisin B1 (27–6140 ppm) and B2 (5–1,550 ppm) even though the fungi were isolated from dairy cattle feed.

*Fusarium proliferatum* is known to cause diseases like *Fusarium* crown and root rot of asparagus (Elmer, 1990), leaf spot of cymbidium (Geriach and Nirenberg, 1982) and rot of garlic bulb (Dugan et al., 2003). In Guntur and Cudapah districts, indicated that the intensity of the rhizome rot of turmeric was high due to *Fusarium solani* (Reddy et al., 2001). Similarly, different forma specialis than the yellows pathogen, *Fusarium oxysporum f. sp. radicis-betae* is reported to be the cause (Harveson and Rush, 1998). *Fusarium culmorum* (W.O. Smith) Sacco has been reported to cause a crown rot of sugarbeet under drought conditions (Hull, 1960). One isolates each of *Fusarium aeuminatum, Fusarium avenaeum, Fusarium solani*, and *Fusarium moniliforme* caused moderate levels of *Fusarium* yellows symptoms. An isolate of *Fusarium aeuminatum* from Colorado previously had been reported to cause yellows type symptoms in sugarbeet (Ruppel, 1991) but *Fusarium avenaeum* and *Fusarium solani* variously have been reported to cause seedling disease postharvest rot (Bosch and Mirocha, 1992) but not typical yellows symptoms.

Variations in cultural and morphological characteristics of *Fusarium oxysporum f. sp. pisi* isolated have earlier also been reported by (Verma and Dohroo, 2003). First reports of *Fusarium* root and stem rot of greenhouse cucumber caused by *Fusarium oxysporum f. sp. radicis-cucumerinum* in Bulgaria (Vatchev, 2007). These cultural and morphological characteristics are identical to those of *F. oxysporum*.
Schlechtend: Fr. f. sp. *radicis-cucumerinum* riginally described by (Vakalounakis, 1996) and later in other works (Punja and Parker, 2000; Cercauskas et al., 2001; Vakalounakis and Chalkias, 2004).

**Pythium species**

*Pythium ultimum* is a ubiquitous soil borne pathogen which causes damping-off and root rot on plants. Each year, *Pythium ultimum* leads to tremendous economic loss. The first *Pythium* sp. reported in the United States was *Pythium ananndrum*, which emerged in 1930s. The isolation of *Pythium ultimum* was first reported by (Wager, 1931) in the Union of South Africa. But the occurrence of *Pythium ultimum* was not recorded as a problem in the U.S. until 1940s when some researchers found that *Pythium ultimum* can infect rhubarb grown in California.

Traditionally, the identification of *Pythium ultimum* depends on the characteristics of the fungus such as morphology of oogonia, antheridia, sporangia. For instance, *Pythium ultimum* produces spherical sporangia that germinate directly, which is distinct from lobed sporangia formed by *Pythium aphanidermatum* that often produce zoospores. Moderate infections can cause plants wilt, reduce plant populations and retard maturation. Severe infections often lead to plants collapse and dead (Allen et al., 2004). Over the last decades, a number of new techniques were developed for detecting *Pythium* species. For example, restriction fragment length polymorphism (RFLP) analysis has been employed to identify *Pythium* at species level. In India, species of *Pythium* recorded are *Pythium vexans* (Bary, 1881). The use of enzyme-linked immunosorbent assays (ELISA) for detection of *Pythium* sp. has also been reported (Yuen et al., 1998). Kageyama et al. (1997) has used a polymerase chain reaction (PCR) technique with species-specific primers to successfully identify *P. ultimum* from damping-off seedlings of Chinese cabbage, cucumber and sugar beet.
**Review of Literature**

*Pythium* species associated with *Pythium* root rot of bean (*Phaseolus vulgaris* L.) in Eastern Africa (Buruchara, 2001). The first studies of *Pythium* species pathogenic to wheat were conducted in Canada and England about 15 years ago (Vanterpool, 1938; Vanterpool and Truscott, 1932). Middleton (1943) reported 10 *Pythium* species associated with wheat in the United State (Sprague, 1946 and Sprague, 1950) added five species to this list. Rhizome rot of turmeric caused by *Pythium aphanidermatum* (Edson) Fitz was first reported in Srilanka (Park, 1934).

The distribution of *Pythium* sp. causing rhizome rot is given hereunder. Mentioned that turmeric was attacked by more than one species of *Pythium* including, *Pythium aphanidermatum* and *graminicolum*. *Pythium graminicolum* has been reported as the causal agent in erstwhile Madras state and South India in India, this disease was first reported from Krishna district of Andhra Pradesh, Thiruchirapally and Coimbatore (Tamil Nadu) (Ramakrishnan and Souminil, 1954). Thus high incidences of *Pythium* induced rhizome rot have been reported during wet years (Rajan and Agnihotri, 1989) implying the role of high soil water content on disease onset and spread. High soil moisture and adequate temperature (25-30°C) prevailing during this period is conducive for disease onset (Sarma, 1994). Cultural characters of *Pythium aphanidermatum* the mycelium of *Pythium* species is colourless, sometimes lustrous and occasionally slightly yellowish- due to abundant oospores or hyphal swelling or grayish-lilac (Van der Plaats-Niterink, 1981).

Severity of *Pythium* disease is more in areas where rainfall is high or heavy clay soils where drainage is impede (Rajan and Singh, 1973). Yellows wet rot Ginger yellows a serious stem and rhizome rot was first described by Simmonds (1955) from Queensland. Later this disease was reported from Hawaii (Trujillo, 1963) and India. During 1984-87, about 40
per cent losses have been reported from Shillai, Rajgarh and Ronhat and Sirmur areas of Himachal Pradesh (Dohroo et al., 1988).

Independent infection by *Fusarium solani* (Martius) Saccardo in Karnataka (Kumar, 1977) and also combined infections of *F. solani* and *Pythium* sp. have been reported (Drojee, 1986; Chauhan and Patel, 1990). Association of *Pythium* sp. and *Fusarium* sp. especially *Fusarium solani* and *Fusarium equiseti* (Corda) Sacc. has been demonstrated (Bhardwaj et al., 1988). In artificial inoculation tests it has been demonstrated that maximum rotting occurred only when *Pythium aphanidermatum* was inoculated first followed by *Fusarium solani* (Joshi and Mathur, 1987). According to Hawksworth et al. (1995) the genus includes 120 species, some of them phytopathogenic, causing fruit, root or stem rot, pre- or post-emergence damping off of seedlings.

**Phytophthora species**

First report of taro (*Colocasia esculenta*) leaf blight caused by *Phytophthora colocasiae* in Nigeria (Bandyopadhyay & Sharma, 2011). Similarly first report of leaf blight of taro (*Colocasia esculenta*) caused by *Phytophthora colocasiae* in Ghana (Omane, 2012). The species was identified by morphological and cultural characters (Erwin and Ribeiro, 1996) as *Phytophthora nicotianae* Breda de Haan. First report of *Phytophthora ramorum* on *Rhododendron* sp. in Serbia (Bulajic et al., 2008). A *Phytophthora* species was consistently recovered by plating rotted tissues of plants showing symptoms onto a selective medium (Masago et al., 1977) and pure cultures were obtained by single-hypha transfers. First report of *Phytophthora ramorum* on *Viburnum bodnantense* in Belgium (De Merlier, 2003).

First report of *Phytophthora ramorum* on Douglas-Fir in California (Davidson, 2002). The disease in focus has been reported from almost all
Review of Literature

betelvine growing countries in the world including Indonesia, Myanmar (Su, 1973), Sri Lanka (Paul, 1939) and Bangladesh (Roy, 1948). Dastur (1926) reported this disease of Pan (*Phytophthora betle* L.) from Durg caused by *Phytophthora parasitica*. Stem portion of *Phytophthora betle* in Ceylon was reported to be attacked by *Phytophthora* sp. (Anonymous, 1928). In India, the disease has been reported from all the betelvine gardens of the country. West Bengal the highest intensity of foot and leaf rot has been recorded in Midnapore and Nadia district (Dasgupta and Sen, 1999).

First report of *Phytophthora ramorum* infecting California red fir in California (Chastagner & Riley, 2010). The extent of losses may vary from 30-100% in case of foot rot and 20% in case of leaf rots, leading to almost total crop failure (Maiti and Sen, 1982; Dasgupta et al., 2000). There is considerable confusion regarding the nomenclature of the species of *Phytophthora* causing disease (s) under consideration. Mc Rae (1928) established the parasitism of *Phytophthora* species. First report of *ramorum* leaf blight and dieback (*Phytophthora ramorum*) on *Camellia* sp. in the UK (Beales et al., 2004). Later morphological study identified *P. nicotianae* var *parasitica*.

The *Phytophthora* species reported to attack betelvine includes *Phytophthora nicotianae* var. *parasitica* (Mc Rae, 1934), *Phytophthora nicotianae* var. *piperina* (Dastur, 1927) *Phytophthora parasitica*, *Phytophthora palmivora* (Maiti and Sen, 1977). Turner (1969) referred all isolates of *Phytophthora* from Southeast Asia as ‘*palmivora*’ type and this was stated to be true for the Indian isolates as reported. Based on existing keys to *Phytophthora* sp all the isolates from Assam were identified as *Phytophthora palmivora* (Butl.). Mohanty (2000) isolated 16 isolates *Phytophthora* from different betelvine gardens of West Bengal and identified the isolates as *parasitica*. A new species of *Phytophthora*
(Phytophthora capsici) isolated and identified as pathogenic to betelvine was reported from Tamilnadu centre of AICRP.

Under warm (25-30°C) and wet condition Phytophthora capsici caused root and crown infection resulting in wilting (Hausbeck and Lamour, 2004). Similarly, common post-harvest fungi Phytophthora capsici has also been found involved in post-harvest rot of some vegetables viz., taro (Colocasia esculenta (L.) Schott), bottle gourd (Lagenaria siceraria (ex Molina) Standley), egg plant (Solanum melongena L.), common bean (Phaseolus vulgaris L.), sponge gourd (Luffa aegyptiaca Mill.) and tomato (Lycopersicon esculentum Mill.). Phytophthora capsici ranks as a top threat to production of Cucurbitaceae, Solanaceae and most recently Fabaceae vegetables (Hausbeck et al., 2008). The incidence of damping off, foliar blight and fruit rot on melon, peppers, pumpkins, squashes and watermelon caused by Phytophthora capsici has dramatically increased in Illinois, USA (Babadoost, 2000a&b). Infection of the plants in the field may occur at any time during the growing season. Early infections caused seedling blight and later infections caused foliar blight, stem lesion, vine rot, fruit rot and root and crown rot (Lee et al., 2001). Phytophthora capsici has been reported as a serious threat to chili production in Pakistan (Naz et al., 2007, Saleem et al., 1999). First report of Phytophthora ramorum infecting grand fir in California (Riley et al., 2010).

New Phytophthora species were described as a result of field analyses and surveys for Phytophthora ramorum, Phytophthora nemorosa E. M. Hansen and Reeser and Phytophthora kernoviae (Brasier et al., 2005). Although Phytophthora nicotianae is one of the most frequent Phytophthora species in ornamental nurseries in Sicily (Pane et al., 2005), this is the first report of this Phytophthora species as a pathogen on C. humilis worldwide. While, Phytophthora kernoviae a similar niche in the
United Kingdom (Brown and Brasier, 2007). *Phytophthora hedraiandra* was found on *Viburnum tinus* during nursery surveys in Minnesota (Schwingle et al., 2007), from *Viburnum* in the Netherlands (De Cock and Lévesque, 2004) and from *V. tinus* in Spain (Moralejo et al., 2006). *Phytophthora lateralis* (Tucker and Milbrath) is a soil-borne plant pathogen that causes cedar root disease in Port Orford cedar trees (*Chamaecyparis lawsoniana*). *Phytophthora ramorum* is a heterothallic organism with two mating types, originally, isolates were found only in Europe (Werres et al., 2001) and isolates only in the United States (Rizzo et al., 2002). In 2003, an isolate that matched the European population was detected on imported European nursery stock in Belgium (Werres and De Merlier, 2003). Also in isolates were detected on nursery stock in Oregon, Washington and British Columbia that matched the European population (Hansen et al., 2003a).

Species new to the United States have also been found, *Phytophthora hedraiandra, Phytophthora pseudosyringae*. *Phytophthora nemorosa* and *Phytophthora pseudosyringae* occupy a similar ecological niche to *Phytophthora ramorum* in the United States (Hansen et al., 2003b).

**SENSITIVITY OF PATHOGENS AGAINST FUNGICIDES (MIC)**

Fungicides have played a significant role in our defense against plant pathogens which account for about 26% of the total crop losses caused by different pests. Indian farmers have been using crude chemical, animal and plant materials save their crops from the onslaught of pests and diseases since Vedic era (Thind, 2005). There are few reports stating the cases of fungicide resistance in developed and under developing countries like U.S.A., Australia, Japan and European Countries. In India also there are very few cases of fungicides resistance (Pan and Sen, 1980; Gangawane and Saler, 1981; Gangawane, 1981; Gangawane and Reddy, 1985).
Variation in the sensitivity of different pathogens in relation to many fungicides has been reported (Jones and Ehret, 1976; Gangawane and Shaikh, 1988; Hollomon, 1981; Kamble, 1991; Bhale, 2002). Annamalai and Lalithakumari (1996) suggested that it is essential to establish the baseline sensitivity for the fungicide against sensitive strain, Brain (1980) considers that heterogeneous population of nuclei consisting of resistant and sensitive nuclei in the isolates might be responsible for variation in the MIC of fungicides. Bhale and Gogle (2008) reported the development of carbendazim resistance in *Alternaria spinaciae* incitant of spinach (*Spinacea oleracea* L.). There was variation in MIC of Ridomil Gold among the five isolates of *Phytophthora palmivora* var. *piperina* on the agar plates (Patil and Kamble, 2011).

Role of fungicides and biocontrol agents in the management of *Fusarium* wilt of chilli was discussed by Singh (2007). Resistant varieties do not fare well under all agroclimatic conditions due to existence of variability in the pathogen (Beckman, 1987). Sen and Kapoor (1974) noticed that a fungicide in carbendazim did not give good control of wilt. Kapoor (2001) has shown compatibility of carbendazim with *Trichoderma* sp. i.e. a single drenching of carbendazim consistently reduced the number of wilted plants of tomato and increased marketable yields significantly (Atkinson and Adamson, 1977). The application of carbendazim reduced the penetration of fungus to the stems and decreased development of vascular discoloration and associated severity of wilting (Channon and Thomsons, 1973).

Higher disease control with combination of systemic fungicides with protectants could be due to additive effect of these fungicides. Jackson et al., (1980) reported that application of copper oxychloride at 2.25 kg/ha gave effective control of *Phytophthora colocasiae* and increased the corm
yield. Various workers (Cox and Kasimani, 1990; Ghosh and Pan, 1991; Bhattacharya and Saikia, 1996; Das, 1997; Aggarwal and Mahrotra, 1987; Andreeva, 1979; Maheshwari et al., 1999) noticed that spraying with Ridomil MZ at fortnightly intervals effectively controlled the disease under field conditions and gave maximum returns. Data on the effect of eupatorium mulch corroborates the findings of Gurung (2001) who reported significant reduction in *Colocasia* blight severity due to mulching with eupatorium leaves and twigs.

In the study, three different fungicides were tested for their fungitoxicity against five fungal pathogens of leafy vegetables namely, *A. brassicae, C. lindemuthianum, F. moniliforme, H. sativum, S. verruculosum* fungi are regarded as one of the chief causative agents of plant diseases (Cambell et al., 2000). Among all tested fungicides the pathogen *F. moniliforme* was found to be most susceptible against tested fungicides. Whereas *H. sativum* was most resistant against tested fungicides. Similar work was previously reported by several workers (Ravishanker and Mamatha, 2005 and Harlapur et al., 2007) Heterogeneous population of resistant and sensitive nuclei in the isolate might be responsible for variation in the MIC of fungicides (Bains et al., 1982).

Similarly variation in sensitivity and resistant of different fungal pathogens to fungicides was reported by several workers (Dekker and Gielink, 1979; Jones and Ehert, 1981 and Sahera Nasreen, 1982). Fungicidal efficacy of carbendazim, captan, benomyl, triadimefon, propiconazole and suggested that systemic fungicide were more effective than non systemic fungicide against *C. fimbriata* Eillis and Halsted (Pandu et al., 1986 and Xiujian et al., 2000). The fungicide mancozeb and captan being recommended for management of diseases like seedling blight of *A. falcataria* (Srivastava and Soni, 1993), leaf spot diseases of *Populus*
Review of Literature

deltoids caused by *Alternaria alternata* (Dey and Debata, 2000), leaf spot and blight of *Syzygium cumini* caused by *Cylindrocladium quinquesepatum* (Mehrotra and Mehrotra, 2000). Integrated management of *Colocasia* (*Colocasia esculenta*) blight was reported by Rana et al. (2007).

Similar results (Biehn and Dimond, 1970) had also suggested that drench with benomyl resulted in control of *Fusarium* wilt of tomato. The improper handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological state of the fruits and vegetables (Wilson et al., 1991). Carrot leaf blight current management options and fungicide update (Beth et al., 2005). Wilt of gladiolus has been successfully managed by the use of carbendazim (Wani et al., 1982).

Furthermore, a mutation in the cytochrome b gene (G143A) in resistant strains of *Alternaria* was characterized (Ma et al., 2003; Ma and Michailides, 2004a&b). Now the resistance appears to be sufficiently common and widespread that azoxystrobin is primarily used only in pistachio orchards where botryosphaeria panicle and shoot blight is the major disease. The application of fungicides with different modes of action in a spray programme, either on a rotating schedule or in mixtures is a generally recommended resistance management strategy (Staub, 1991; Brent, 1995). Whereas resistance to strobilurins has been demonstrated extensively in previous studies for several fungicide-pathogen combinations, including *Alternaria* of pistachio (Chin et al., 2001; Wong and Wilcox, 2002; Michailides et al., 2005; Pasche et al., 2005; Malandrakis et al., 2006) the identification of *Alternaria alternata* isolates resistant to the new anilide fungicide boscalid is a particularly new phenomenon.
Review of Literature

This is the first report where a fungal pathogen has been shown to be resistant to boscalid although Zhang et al. (2007) obtained boscalid-resistant mutants of *B. cinerea* following *in vitro* UV treatment. Several studies reported the effects of boscalid on some fungal pathogens of important crops, but they were only conducted to establish baseline sensitivity values to boscalid or reveal its efficacy (Lu et al., 2004; Matheron and Porchas, 2004; Spiegel and Stammler, 2006; Stammler and Speakman, 2006).

In the event of high disease risk due to congenial weather conditions, phenylamide fungicides, notably metalaxyl-based formulations are frequently used by the farmers in India to get effective control of the disease (Thind et al., 2001). Importance of mixtures of specific action fungicides with multisite compounds in managing potato late blight and in preventing resistance build up to risky molecules has earlier been highlighted by Thind et al. (2004). The potential of several of these new fungicides has been documented in a recent review (Stevenson, 2009).

Use of fungicides is the best method of controlling the diseases whenever there is outbreak of disease. Mukewar and Gera (1980) tested nine fungicides in the laboratory for inhibition of the conidial germination of *Alternaria helianthi*. They reported that Dithane M-45 and Vitavax were more effective followed by Bavistin and Benlate. Singh and Milne (1974) evaluated the efficiency of 15 fungicides against five fungi causing chrysanthemum flower blight, viz. *Alternaria alternata*, *Botrytis cinerea*, *Itersonilia perplexans*, *Mycosphaerella ligulicola* and *Stemphylium vesicarium*.

combi product Iprodione Carbendazim as effective fungicide against *Alternaria helianthi*. Mathur et al. (1971) reported that Dithane M-45 was better than Dithane Z-78 and other fungicides tested against *A. solani* of potato. Out of the 10 fungicides tested against *A. tenuissima*, Thiram, Duter, Captan and Dithane M-45 inhibited the growth of the fungus *in vitro* studies. At higher concentration of 0.4 per cent, Dithane M-45 was superior to Captan, Thiram and Duter (Hanumanthaiah, 1976).

Basavarajaiah et al. (1979) found that the fungicides Thiram, Duter, Brestan, Aureofungin and Captan in decreasing order were effective against *Alternaria carthami* *in vitro* while, Mahabaleshwarappa (1981) reported that Baycor caused the good inhibition of *Alternaria carthami* under *in vitro* followed by Deconil, Hexafero, Blitox and Cumin-L. Padmanabhan and Narayanasamy (1976) and Padaganur and Siddaramaiah (1979) reported that, Dithane-Z-78 and Brestan, Duter and Hexafero were more effective against *Alternaria macrospora* respectively. Natarajan (1980) conducted both *in vitro* and *in vivo* fungicidal trials using several fungicides to control *Alternaria* leaf blight of sesame and obtained maximum control with Dithane M-45 followed by Dithane Z-78, Duter, Captan and Thiram. Joshi (1981) and Hadagali (1981) reported that Rh-2161 a systemic fungicide gave the best control of *Alternaria gompherenae* and *Alternaria longissima* respectively.

Hiremath and Sundaresh (1985) reported that in fungicide tests against *A. tenuissima*, thiram effectively inhibited the fungal growth *in vitro* and Mancozeb and Thiram gave excellent control *in vivo*. In *in vitro* evaluation of eight fungicides against *A. alternata* causing leaf blight of turmeric, Propiconazole (tilt) was found to be superior in inhibiting the growth of the fungus while Ziram a non systemic fungitoxicant found to be the best in inhibiting the growth of fungus (Mallikarjun, 1996). Hwang et
al. (1998) reported that among the three fungicides tested (Mancozeb, Iprodine and Pyrifenoxy) Pyrifenoxy was the most effective in inhibiting mycelial growth of *Alternaria alternata* on water agar. Kamble et al. (2000) tested six fungicides against *Alternaria alternata* under *in vitro* conditions. They reported that Mancozeb was highly effective in inhibiting the mycelial growth followed by Copper Oxychloride and Iprodine at 1000, 2000 and 3000 ppm. Urbanszki et al. (2003) tested *in vitro* the efficacy of 16 fungicides against *A. alternata*. They reported that Tridemorf fungicides proved to be very efficient in controlling the pathogen.

**SYNERGISTIC EFFECTS**

The purpose of this study was to determine the occurrence of synergistic antifungal interactions between a number of fungal hydrolases and different classes of fungitoxic compounds and to determine if the level of synergism may be related to the mechanism of action of the toxins used. Synergistic effects of combining biocontrol agents with silicon against postharvest diseases of jujube fruit (Shiping et al., 2005). The combination of biological control agents with selected chemicals produces a synergistic effect that enhances their efficacy for postharvest disease control (Droby et al., 2003).

However, these antagonists of fungal pathogens, especially under semicommercial conditions usually are not as effective as chemical biocontrol antagonists with salicylic acid (Qin et al., 2003).

The use of synthetic chemicals as fungicides is a primary method of control of disease-causing fungi in animals including humans and crop plants. However, the exposure of human populations and natural habitats to increasing amounts of pesticides is becoming unacceptable and new strategies are required in the attempt to eliminate or reduce the doses of chemicals needed (Cook & Granados, 1991). The chemicals chosen had
different modes of action (Koller, 1992; Jones & Hancock, 1988; Miller 1969; Ishii, 1992); thus the ability of the enzymes to synergistically interact with the pesticides tested was not associated with a single class of compounds.

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES

Brannon (1923) observed that glucose and fructose were utilized equally well by *Fusarium* sp. for spore formation when grown on Czapek’s modified solution. Sebek (1952) obtained maximum quantity of mycelium of *F. oxysporum* f. sp. *lycopersici* and *F. vasinfectum* in the presence of 10 per cent glucose and 2.5 to 5 per cent xylose respectively. Kesavan and Prasad (1975) reported that among the carbon sources tested glucose and sucrose were found to be the best for the production of fusaric acid and sporulation by the muskmelon wilt pathogen while starch provided maximum growth.

Brayford and Bridge (1989) reported growth and pigmentation of 99 strains of *Fusarium* mainly *F. oxysporum* and *F. solani* on ammonium salts agar containing manitol, sorbitol or xylitol as sole source of carbon. Patel (1991) demonstrated that, among carbon sources, maltose and mannitol were best utilized by *F. solani*. Moore (1924) reported that potassium nitrate, asparagine and ammonium salts of certain organic and inorganic acids served as nitrogen sources for *Fusarium coeruleum* whereas oxalates and formates did not support its growth. Moore and Chupp (1952) a physiological study of the *Fusarium* causing tomato cabbage and muskmelon wilts.

Subramaniam and Pai (1953) reported good growth of *F. vasinfectum* on potassium nitrate, while ammonium sulphate was found to be poor source of nitrogen. Chi and Hanson (1964) found variation among three isolates of *F. oxysporum* and two isolates of *F. solani* from red clover
with respect to nitrogen and temperature requirements. Bhatnagar and Prasad (1968) showed good growth of *F. solani* in D-leucine and asparagine whereas asparagine and non-valine proved to be good for 11 isolates. However, sodium nitrate did not support any growth of both the isolates.

All the isolates of *F. oxysporum f. sp. niveum* preferred nitrate to ammonical nitrogen. The fungus may convert certain forms of complex carbon compounds into simple form, which may be readily metabolized (Bais et al., 1970). Maximum growth was obtained on potassium nitrate, ammonium oxalate, ammonium sulphate and ammonium phosphate (Selvaraj, 1971). Kushwaha et al. (1974) working with eight strains of *F. oxysporum f. sp. lentis* for nutritional requirement noticed variation with regard to utilization of nutrients. Mahendrapal and Grewal (1975) showed that ammonium salts in general supported growth of *F. oxysporum f. sp. ciceri.* Its growth was meager on calcium nitrate, whereas moderate to good growth of the fungus was recorded on monosodium dicarboxylic acids and amides.

Hydrolysis of oligosaccharides results in the formation of mono and disaccharides, which are readily utilized maltose as preferential carbon source to pathogenic fungi like *Alternaria* sp (Goyal, 1973). Tandon and Chandra (1992) also reported the preference of maltose over glucose by fungi like *Alternaria alternata* and *Fusarium oxysporum.*

Nitrogen being essential element fungi requires nitrogen in same form or other however preferential source vary in different fungal types (Dansentos, 1963). Addition of biotin is reported to increase the growth of several fungi like *Botryodiplodia theobromae* and *Fusarium monoliforme var. subglutinas* (Mitra and Lele, 1981) and *F. oxysporum* (Patel and Patel, 2000). Gangawane and Reddy (1986) indicated that micronutrients such as
Cu, Mo, Mn, Al and Co in combination with cabendazim not only decreased resistance but also delay the latent period in vitro of *Aspergillus flavus*. Increased production of amino acid in mercury and capton resistant isolates of *Macrophomina phaseolina* have been noted by Rana and Sengupta (1976).

Sahera Nasreen and Dabhadkar (2000) reported that physiological and biochemical characteristics of sensitive and resistant *Fusarium oxysporum f. sp. lycopersici*. Isolates were influenced by nutrient sources. Among the carbon and nitrogen sources tested, maltose and casein hydrolysate and form amino acids and vitamins tested, asparagin and biotin supported maximum growth and pycnidial production of *Phomopsis theae* (Ponmurugan and Baby, 2007).

Biochemical changes were observed in spinach infected with carbendazim resistant *Alternaria spinaciae* (Bhale et al., 2010). There are many reports indicating the biochemical characteristics in different host (Mayee and Chakraborty, 2004) infected with pathogens. Siddaramaiah and Hegde (1990) studied on change in biochemical constituents of *Cercospora* infected mulberry leaves and found that infection by the pathogen induced changes in the chemical constituents like total amino acids, phenol and sugars. Sundares et al. (1988) reported that diseases leaves are biochemically poor in nutritive value and indicated the reduction of moisture, protein and sugar contents. It is reported that total sugar, reducing sugar, non-reducing sugar and starch contents of mulberry leaves were found to be decreased with the increase of disease intensity (Ali, 1995 and Ghosh, 1996). Similar results have been reported in fruit rot disease infected guava by Saud et al. (2000). Naik and Hiremath (1988) reported that the total suger content decreased in *Colletotrichum gloeosporioides* infected betelvine leaf. Increase in free amino acids may be due to
proteolysis of fruit proteins catalyzed by the fungal enzymes (Arya, 1993). These differences may be due to deterioration caused by the fungi since fungi require some essential nutrients for growth of survival (Ogbonna et al., 1998; Campbell, 1985).

ENZYMES AND TOXINS

Plant pathogens is the production of extracellular enzymes such as the most important factor for Cellulases, Pectinases, Amylases, Lipases and Chitinases by the *Alternaria tenuissima*, *A. spinacia*, *A. alternaria*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Fusarium oxysporum* (1) *Pythium* sp. and *Phytophthora colocasiae*. Saprophytic fungi produce strictly regulated amounts of enzymes in order to digest cellulose and to use it as the sole carbon source (Mendgen and Deising, 1993). Members of the tested fungal genus have been extensively studied particularly due to their ability to secrete cellulose degrading enzymes or to act as biocontrol agents.

Lilly and Barnett (1951) also reputed antolysis after maximum growth where cellular enzymes begin to digest the various cell constituents. Similar findings were also observed by Ekbote (1994), Hiremath et al. (1993) in case of *C. gloeosporioides*. Most phytopathogenic fungi are capable of producing polysaccharide degrading enzymes which can alter or degrade a number of the polymeric carbohydrates found in higher plant cell walls (Mussell, 1973; Cooper and Wood, 1980; Barash et al., 1984; Bahkali, 1983& 1992; Aljohimi, 1995). Some pathogens produced both pectic hydrolases and lyases (Goodenough and Maw, 1974; Bahkali, 1987), while others produce one of these enzymes (Bateman, 1972).

The induction of cellulolytic enzymes by crystalline cellulose or soluble derivative molecules which cannot enter the cell is held to be mediated by low molecular weight cellulose degradation products or their transglycosylation products (Messner et al., 1988). Cellobiose (Mandels
and Reese, 1960; Khiyami, 1994) and 13-methylglucoside (Sternberge and Mandels, 1979) were used as the sole carbon source. In culture the production of hydrolytic enzymes by many fungi requires substrate and is repressed by preferred carbon sources such as glucose (Cooper, 1976; Collmer and Keen, 1986; Keon et al., 1987). As pecificity of glycoside hydrolases has also been observed in other fungi; fl xylosidases from Trichoderma reesei (Poutanen and Puis, 1988) and from Neurospora crassa Desphande et al.(1986) exhibit a-arabinosidase and flglucosidase activities, respectively. Cell wall which is composed of pectin, polysaccharides, cellulose, hemicellulose, lipids and proteins is degraded by the enzymes produced by a virulent pathogen (Wheeler, 1975). There are reports where more enzymes are produced by highly virulent isolates of Pythium stalk rot of corn than the weakly virulent ones (Sadik et al., 1983). The study of slow moving pathogens and their production of cellulases have also been reported by Wood (1960). Stalk rot of corn is significantly correlated with cellulase production (Chambers, 1987).

Like proteases, membrane degradation by lipase depends on the involvement of specific type of enzyme and membrane (Condrea and de Vries, 1965). The disease causing organisms enter the host tissue through mechanical pressure exerted by the growing germ tube or dissolving the host cell wall through secretion of toxins or enzymes (Albersheim et al., 1969). The plant cell wall is a complex structure of polymers which surrounds the cell containing cytoplasm (Karr and Albersheim, 1970). Apart from cell wall degrading enzymes secreted by a wide variety of saprophytic and phytopathogenic micro organisms (Bailey and Pessa, 1990; Rombouts and Pilnik, 1980), the pectinases also play an important role in the entry of the plant pathogen intra and intercellularly into the host tissues thus blocking the conducting vessels resulting in the development of wilt (Soni and Bhatia, 1981).
Loss in germination could be indicative of severe damage caused by aflatoxin B1 to cell membrane (Banerjee et al., 1990; Crisan, 1973) or due to production of cell wall degrading enzymes (Fawole et al., 2006) as well as reduction in seedling amylase activity (Hasan, 1999).

These pre-harvest and post-harvest mycoflora not only cause seed deterioration but also make seed unfit for human consumption (Miller, 1995). In many cases the fungi affect the seeds during storage through the production of toxic metabolite (Turner, 1971). There are many reports on seed mycoflora of coriander and their effect on seeds (Vaidehi, 1984; Prasad, 2004). Reduction in seed germination and radicle growth of coriander due to the harmful effect of secondary metabolites of *Fusarium* (Hashim and Thrane, 1990).

Inhibitory factor present in the fungal culture filtrate may be responsible for these adverse effects on seed (Tiwari, 1993). Mycoflora of seeds have high protolytic and cellulolytic enzymes beside the power of dissolving cutin (Prasad, 1979). Fungal metabolites not only affect seed health, cause damage in seedling, enhance disease incidence in later stage of plant but also affect consumers. (Bateman and Kwasna, 1999).

The above results conformed the findings of Vidyasekaran et al. (1970). The production of secondary metabolites by fungi is known to degrade seed quality and reduce the seed viability (Caster and Frederikson, 1980; Gopinath and Shetty 1988). The similar results were also observed by Arun and Mathew (1991), Gachande and Jadhav (2010) in case of seeds of pigeonpea, gram varieties respectively. Mycotoxin production by *Fusarium proliferatum* isolates from rice with *Fusarium* sheart rot disease was evaluated by (Abbas et al., 1999). The producers of the toxins, mainly *Fusarium moniliforme* and *Fusarium proliferatum* have been found
frequently in corn and corn-based products or in barley and wheat (Nagaraj et al., 1996; Torres et al., 2001).

Soybean seeds soaked in culture filtrates of *Fusarium soloni, F. oxysporum, Aspergillus flavus, A. niger, Alternaria tenuis* and *A. alternata* for 24 hours showed reduction in percentage of seed germination was observed by Ibraheem et al. (1987). Filtrate from mycelial cultures of *Verticiilium alboatrum* was found to inhibit cell growth and reduced the viability of alfalfa seeds (Frame et al., 1991). Abrahum (1978) was also reported the inhibitory effect of culture filtrates of fungi on seed germination.

**ECO-FRIENDLY MANEGEMENT**

Secondary metabolites are antimicrobial in nature and are produced by plants as a part of natural defense system against pathogens and predators. This property of plants is being exploited for use as biological control. Demand for natural pesticides and fungicides are increasing day by day therefore it is necessary to investigate plant for their effectiveness as antifungal agents for possible use against pathogenic fungi. Therefore, the present investigation was undertaken to evaluate different native bioagents leaf extract, plant latex, essential oil and *Tricoderma* sp. under *in vitro* conditions.

**Leaf Extract**

Exploitation of plant metabolites in crop protection and prevention of deterioration caused by fungi appear to be promising. Sheikh and Agnihotri (1972), Kaushal and Paul (1987) demonstrated strong fungitoxic activity of eupatorium against the growth of various pathogens. Shivpuri et al. (1997) observed that ethanol extract of *Azadirachta indica, Datura stramonium, Ocimum Sanctum, Polyalthia longifolia* and *Vinca rosea* were more toxin to *Alternaria brassicicola, Colletotricum capsici, Fusarium oxysporum,*
Rhizoctonia solani and Sclerotiana sclerotium. Similarly Dubey (1998) found that root extracts of Moringa oleifera inhibited the growth of Thanatophrous cucumeris followed by Ficus and Eclipta leaf extracts.

Bhowmick and Chaudhary (1982) recorded that the leaf extract of Acalypha indica completely checked the growth of Alternaria alternata. Bisht and Khulbe (1995) also reported that plant extract of D. stramonium inhibit the mycelia growth of H. sativum. Kotasthane and Lakpale (1994) found that extracts of Oryza sativa, Phalaris minor and Lycopersicon esculantum. Traditionally plant fungal diseases are controlled by synthetic fungicides which increase agricultural costs and contaminate the environment with very toxic substances (Silva et al., 2008). Efficacy of fungicides, bioagents and plant extracts against pink root rot disease of onion induced by Fusarium solani (Mathur, 2007). Many researchers have reported on the fungal inhibitory property of Azadirachta indica. Fusarium solani leaf blight in Terminalia cateppa (Mamatha and Rai, 2004), conidial germination of powdery mildew fungi (Nair and Arora 1996) Sclerotium rolfsii (Singh and Dwivedi 1990) have been shown to be inhibited on treatment with neem extract.

Presence of Calotropis aqueous extracts which lowered the percentage germination of Penicilium lanosum after 8 hours without affecting the tube length and growth Calotropis procera proved to be phytotoxic and antimicrobial against soil borne fungi, Gram+ve and Gram-ve bacteria (Hassan et al. 2006; Awadh et al., 2001; Usha et al., 2002). In contrast Calotropis procera extract has no effect on chickpea wilt cause by Fusarium oxysporum f. cicers (Chand & Singh, 2005).

Leaf extracts of various plants are known to possess antimicrobial activity. Antimicrobial activity of the leaves has been mentioned by Charjan (1995), Abd-Aziz et al. (1994-1996), Suhaila et al. (1996) etc. The
Review of Literature

toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods which are eco-friendly and effective (Mohana et al., 2011). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Parekh et al., 2006; Aliero and Afolayan, 2006; Buwa and Staden, 2006; Ergene et al., 2006). In view of these, the author screened some leaf extracts against seed-borne pathogenic fungi and the data has been presented in this paper.

Plant Latex

India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as source of new drugs is an innate and very important component of healthcare system. Although it appears to be a great array of antifungal drugs there is at present a quest for new generations of antifungal compounds due to the low efficacy, side effects or resistance associated to the existing drugs.

The antifungal activities of fractions were assayed with the method of agar incorporation “dilution in a solid medium” (Eloff, 1998) including negative control as previously described (Bel Hadj Salah et al., 2007). Minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using 96 Well microliter plates (Mitscher et al., 1972). The MIC was defined as the lowest concentration of the compounds to inhibit the growth of micro-organisms (Karaman et al., 2003). The inoculums were prepared overnight (24h at 37° C) cultures in Muller Hinton broth medium (MHB). Petri dishes were inoculated with suspensions of 106 germs/ ml (Rio et al., 1988).
The antifungal potency of *C. gigantea* latex extract on the *C. albicans* showed a larger diameter of clearance than that of other fungal strains (Venkatesan and Subramanian, 2010). The latex extract were screened *in vitro* against human pathogenic strains such as Gram positive; *Staphylococcus aureus, Bacillus subtilis*, Gram negative; *Salmonella typhi, Klebsiella phenonemia* and two fungal strains; *Aspergillus niger* and *Candida albicans*. The result agrees with that there is a need to employ broad range of extractive solvents in the extractions of possible photochemical from medicinal plants (Takazawa et al., 1982). The growth of four test fungi were inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The mycelial growth, percentage spores germination and germ-tube extension in *Fusarium oxysporum* and *Aspergillus carbonaris* decreased when *Calotropis procera* extract concentration increases, where as growth of *Humicola brevis* and *Penicillium lanosum* were not affected (Rizk, 2008).

The water-soluble fraction of papaya latex can completely digest the conidia of many fungi including important post harvest pathogens (Indrakeerthi and Adikaram, 1996). Other latex extracted from several plants showed a strong antifungal activity against *Botrytis cinerea, Fusarium* sp. and *Trichoderma* sp. (Barkai-Golan, 2001). The best antifungal activity was recorded in ethanol extract of *C. procera* latex against *Candida albicans* (Kareem et al., 2008). Leaf extracts, chopped leaves and latex of *C. procera* have shown great promise as a nematicide *in vitro* and *in vivo* (Khirstova and Tissot, 1995).

The latex extract has also been qualitatively analyzed for the presence of different phytochemicals. It evaluated the wound healing and antibacterial activity of *C. gigantea* latex (Saratha et al., 2009; Subramanian and Saratha 2010). In addition the traditional medicine related
to treatment of both human and animal mycoses with plant-derived preparations is considered a valuable knowledge for the discovery of new antifungal drugs (Nwosu, 1995). Plants contain many biologically active molecules with different medicinal properties (Newman, 2003; Butler, 2004). This plant is popularly known because it produces large quantity of latex. Latexes are source of various biologically active compounds, including glycosides, tannins and many proteins, among others (Wititsuwannakul et al., 2002; Dubey and Jagannadham, 2003). Previous work, using different parts of the plant has advocated its use for a variety of disease conditions (Basu and Chaudhuri, 1991) in addition to the application as an antidote for snake poisoning. Fractionation of the latex into its rubber and rubber-free fractions affords better insight into its potentials and limitations (Ramos et al., 2006).

**Essential oils**

Chemical and biological studies are useful to understand and appreciate biodiversity. In general, isolating, identifying, and determining structures of new metabolites are fundamental to reveal their chemical potential a first step to use, conserve and protect them (Castillo, 1992). According to Niemeyer (1995) about 5% of all 5971 known species of Chilean flora (Marticorena, 1990) have been chemically studied. Moreover there is great interest to replace synthetic xenobiotics with similar acting natural compounds. It is important to determine secondary metabolites with fungicidal or fungistatic activity since they allow the use of natural origin compounds that are generally species specific, have low environmental persistence and are biodegradable.

Of the main terpenes found in the essential oils of *P. boldus*, *L. philippiana* and *L. sempervirens* the components ascaridole, 3-carene, and α-phellandrene were previously described for *P. boldus* (Gupta, 1995;
Review of Literature

Schrickel and Bittner, 2001; Montes et al., 2001) and safrole for L. sempervirens. The results of this study concur with the research cited above, but also revealed safrole in L. philippiana and 1,2-dimethoxy-4-(2-propenyl)-phenol was found exclusively in this species. Eucalyptol was ascertained in P. boldus and L. philippiana essential oils. Finally, L. philippiana revealed the presence of α-1-methyl-3-cyclohexadiene, previously described for Persea lingue (Ruiz and Pav.) and Nees, a member of the Lauraceae family which is close to the Monimiaceae family (Marticorena and Rodriguez, 2001).

The uses of plant-derived products as disease control agents have been studied since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee et al., 2007). Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants (Isman, 2000). They were previously known to have biological activities such as antifungal (Soliman and Badeaa, 2002), antibacterial (Dorman et al., 2000), insecticidal and nematicidal effects (Pandey et al., 2000).

The essential oil from the leaves of H. suaveolens has been reported earlier by Saxena et al. (1978). Earlier the antifungal and antimicrobial activity of H. suaveolens oil was reported by Iwu et al. (1990) and Pandey et al. (1982). The most important soilborne of fungi, P. ultimum and R. solani, had 100% complete inhibition of mycelial growth and agreement with those obtained by Huv et al. (2000) that showed Eucalyptus unigera oil inhibited mycelial growth of three phytopathogenic fungi such as C. gloeosporioides, R.solani and Pythium sp. These compounds, especially carvacrol, borneol, camphor and anethole have been reported to inhibit the growth of Phytophthora infestans. Sporangial production was inhibited by the essential oil of fennel (Soylu et al., 2006).
Bittner et al. (2008) previously tested the effect of essential oils from *Gomortega keule* (Molina) I. M. Johnst., *Laurelia sempervirens*, *Origanum vulgare* L., *Eucalyptus globulus* Labill and *Thymus vulgaris* L. on the *Sitophilus zeamais* and *Acanthoscelides obtectus* (Coleoptera) granary weevils obtaining promissory results that suggest their use in grain storage pest control.

Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. Bioresour (Wang et al., 2005). Alternative low cost effective and species-specific control methods should be found that do not leave permanent toxic residues in the environment. Previous studies of essential oils from aromatic plants such as *Ocimum canum* Sims and *Citrus medica* (L.) (Dubey et al., 1983), *Pimpinella anisum* (L.) (Shuklay Tripathi, 1987), *Cinnamomum camphora* (L.) J. Presl (Mishra et al., 1991), *Cymbopogon citratus* (DC.) Stapf (Mishra and Dubey, 1994) and *Chenopodium ambrosioides* L. (Mishra et al., 2002) have demonstrated their strong fungicidal activity.

The 1, 2-dimethoxy- 4-(2-propenyl)-phenol compound, known to be one of recognized toxic activity was found only in *L. philippiana* and could be attributed to fungistatic activity (Pérez and Ubera, 2006). According to Niaz and Kazmi (2005) neem oil was quite effective for *Aspergillus* sp. Vir and Sharma (1985) found antifungal activity in neem oil against *Alternaria alternata* and *Aspergillus* sp. Sinniah et al. (1983) also studied the toxicity of neem oil on *Aspergillus* sp.

**Biocontrol agent (Trichoderma sp.)**

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular pathogens cause important losses fungi being the most aggressive. Chemical compounds have been used to
control plant diseases (chemical control) but abuse in their employment has favored the development of pathogens resistant to fungicides (Tjamos et al., 1992). By contrast the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists (Monte, 2001).

A considerable role in limiting the populations of these pathogenic fungi inhabiting the above ground parts of plants is played by antagonistic microorganisms. Such properties are first of all exposed by the fungi Trichoderma and Gliocladium (Massart and Jijakli, 2007; Sempere and Santamarina, 2007; El-Katatny et al., 2006; McQuilken et al., 2001; Roco and Perez, 2001; Ahmed et al., 2000; Harman, 2000; Kredics et al., 2000; Elad and Kapat, 1999; Gupta et al., 1999; Yedidia et al., 1999; Lumsden et al., 1992; Lifshitz et al., 1986).

Therefore, it may be more prudent to search for biological antagonists against specific pathogen and evaluate blends of antagonists for wider applications (Baker and Cook, 1974). These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the strain the antagonized fungus the crop plant and the environmental conditions including nutrient availability pH, temperature and iron concentration (Bell et al., 1982).

Application of the fungicides is not economical in the long time because they pollute the environment leave harmful residues and can lead to the development of resistant strains of the pathogen with repeated use (Vinale et al., 2008). Replacement of fungicides with bio-control agents is an alternative mean to manage the plant pathogens produce safety food and reduce the environment pollution (Barakat and Al-Masri, 2005). One of the most important biocontrol agents is Trichoderma sp. that the most frequently isolated soil fungi and present in plant root ecosystems.
Trichoderma sp. also are commercially marketed as biopesticides biofertilizers and soil amendments. The use of Trichoderma fungi in agriculture can provide numerous advantages; 1) Colonization of the root and rhizosphere of plant, 2) Control of plant pathogens by different mechanisms such as parasitism, antibiosis production and induce systemic resistance, 3) Improvement of the plant health by promote plant growth and 4) Stimulation of root growth (Harman et al., 2004).

The antagonistic activity of the genus Trichoderma to F. solani and R. solani has been widely demonstrated (Lewis et al., 1998). Application of T. harzianum as seed treatment significantly reduced the incidence of damping-off diseases some leguminous crops i.e. faba bean, lentil and chickpea when planted in a soil naturally infested with Fusarium sp. and R. solani (Abou-Zeid et al., 2003). T. harzianum, Trichoderma koningii and T. viride as seed dressing improved the seedling emergence and health of runner bean (Phseous coccineus cv. Eureka) (Pieta et al., 2003).

Soil provides the medium for root development and with the exception of carbon, hydrogen, oxygen and some nitrogen plants depend on soil for all other nutrients and water. The soil microbes that include bacteria, fungi, actinomycetes, protzoa and algae play a significant role in the nutrient cycling (Nannipieri et al., 2003). Interactions between plant root systems and bio-control agents such as rhizobacteria are able to generate a wide array of secondary metabolites which can have a positive influence on plant growth, enhancing the availability of minerals and nutrients, improving nitrogen fixation ability and improving plant health through the biocontrol of phytopathogens (Sturz and Christie, 2003). Bacillus subtilis and T. viride only or combined were significantly increased the values of NPK concentration on tomato plants compared to control treatments (Henry et al., 2009 and Morsy et al., 2009).
Nawar and Kuti (2003) reported that there are positive relationships between peroxidase and resistance development in plants. Caruso et al. (2001) also experimentally supported the idea that peroxidase play a defense role against invading pathogens. Hassan et al. (2007) recorded the lowest percentages of chocolate spot disease severity and the highest levels of peroxidase activities.

*Trichoderma* is listed both in Europe and USA as an active principal ingredient permitted form use in organic farming for plant disease control. *Trichoderma* sp utilize various mechanisms including nutrient competition, antibiosis and antagonism inhibition of pathogen or plant enzymes processes of biodegradation, carbon and nitrogen cycling; complex interactions with plants in the root zone of the rhizosphere which involve various processes such as colonization plant growth.

Stimulation of bio-control of diverse plant pathogens, decomposition of organic matter symbiosis and nutrient exchange (Howell, 2003 and Harman, 2006). Recent studies indicate that these fungi can induce systemic resistance in plants, thus increasing the plant defense response to diverse pathogen attack (Harman et al., 2004).

Several workers have been reported that the use of *Trichoderma* species against number of plant pathogenic fungi (Brisa et al., 2007; El-Mougy et al., 2007) Akbari and Parakhi (2007) reported *T.viride*-I and *T.hamatum*-IVandV isolates showed strong antagonism against *Alternaria alternata* causing blight of sesame. High inhibitory effect of volatile toxic substances emitted by *Trichoderma* sp on the radial growth of *Fusarium* sp has also been reported by Dubey et al. (2007). The inhibition was high with the direct use of *Trichoderma* spp. in dual culture against *Fusarium oxysporum f. sp. Psidii* (61-69%) and *Fusarium solani* (58-68%) (Gupta and Mishra, 2009). Kumar et al. (2007) tested three species of *Trichoderma* i.e. *T.virens, T. viride* and *T. harzianum* against *F. moniliforme var subglutinans* and found them effective.