Chapter - II

Review of Literature
REVIEW OF LITERATURE

Pre and Post-harvest diseases caused by bacteria, yeast and fungi develops on fruit and others plant products between harvesting and consumption. Post-harvest diseases destroy 10-30% of the total yield of crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield (Agrios, 2005; Kader, 2002). Fungi are the most important and prevalent pathogens, infecting a wide range of host plants and causing destructive and economically important losses of most fresh fruits and vegetables during storage and transportation (Sommer, 1985).

Today, there is a growing movement in many countries to reduce the amount of chemicals being released into the environment. Among the notable hazards of misuse and overuse of pesticides are the induction of resistance in plant pathogens, occupational hazards, environmental pollution, destruction of natural enemies (ecological hazards) and residues in food which are poisonous and/or carcinogenic, mutagenicity, genetic damage and teratogenicity, acute and chronic residual toxicities and severe and acute mammalian toxicities. Due to their residual nature these synthetic chemicals can adversely affect a number of biological systems. With regard to acute toxicity, fungicides are reported to be less hazardous compared to insecticides and herbicides, though the mercurial compounds could be exceptional in this instance, and they are gradually being replaced. Some fungicides are known to have phytotoxic side effects. Specifically acting fungicides have been observed to cause pathogen resistance. One example of this is the complete failure of the fungicides (benomyl, thiabendazole, carbendazim thiophanate methyl) due to the development of resistance in a number of pathogenic fungi. Presently, such alternatives seem to come from natural plant products which are
known to be of low mammalian toxicity and are highly biodegradable. The use of such pesticides in Pest control strategies could result in an increased of the amount of synthetic pesticides with positive effects on the environment (Englelska and Radgivare, 1995).

In the hierarchy of man's need, the provision of substantial amount of food and products to the increasing population comes the foremost. Demographic estimates indicate the huge leap in the world population to about 6200 million in 2000 A.D., thus requiring an increase in agricultural production of about 50-60 percent (Buchel, 1983). The present food grain production is around 199 million tonnes (GOI, 1998) and it is expected that by 2000 A.D. India would require about 220 million tonness of food grains. In today's intensive agriculture, the pest and disease problems have been made worse by practices such as cultivation of high yielding varieties, increased use of irrigation and fertilizers, greater number of plants per unit area or per unit time.

The incidence of various diseases attacking crop plants is likely to increase further under intensive agricultural operations (Sokhi et al., 1992). In comparison to fungicides are the smallest groups of pesticides but they show a good growth rate and future potential probably due to the recent introduction of fungicides in field crops that were not treated with them before.

There are nearly 155 different fungicidal compounds in international markets. When Horshfall published a book, "fungicides and their action" he had not mentioned resistance of plant pathogens to fungicides. But later on in 1956 he included a topic on acquired fungicidal resistance in plant pathogens in that book. Later on (Georgopoulos and Zarcovitis, 1967) reported the tolerance of fungi to organic fungicides in "Annual Review of Phytopathology." Fungicide resistance management (Dekker, 1982; Brent, 1987; Schwinn and Morton, 1990; Staub, 1991
were drawn upon freely in the original preparation of this monograph and are still of considerable value.

Some agrochemical companies have also tended to use 'in sensitivity', 'loss of sensitivity' or 'tolerance', because these sound less alarming than 'resistance' on the other hand, two studies on terminology recommended that 'resistance' should be the preferred term (Anon, 1979; Delp and Dekker, 1985). With the introduction of the systemic fungicides, the incidence of resistance increased greatly, and the time taken for resistance to emerge was often relatively short, sometimes within two years of first commercial introduction. Many of the fungicides introduced since the late 1960s have been seriously affected, with the notable exceptions of the amine fungicides ('morpholines'), fosetyl aluminium, anilinopyrimidines phenylpyrroles and some of the fungicides used to control rice blast disease (e.g. probenazole, isoprothiolane and tricyclazole), which have retained effectiveness over many years of widespread use.

Some recently introduced fungicides such as benzamides and carboxylic acid amides have not yet encountered serious resistance problems, possibly because of the management precautions which have been taken. Most of the older material such as copper fungicides, sulphur, dithiocarbamates (e.g. mancozeb), phthalimides (e.g. captan) and chlorothalonil, have retained their full effectiveness in all their uses, despite their uses despite their extensive and sometimes exclusive use over many year. Once developed, it tends to be stable. If the fungicide concerned is with drawn or used much less, pathogen populations can remain resistant for many years; a well documented example is the sustained resistance of *Cercospora betae*, the cause of sugarbeet leaf spot, to benzimidazole fungicides in Greece (Dovas et al., 1976). A gradual recovery of sensitivity can sometimes occur, as in the resistance of
*Phytophthora infestans*, the potato late blight pathogen, to phenylamide fungicides (Cooke et al., 2006).

**Isolation and pathogenicity of the pathogens**

**Isolation**

Isolation and purifications the pathogen, *Bipolaris oryzae* was isolated by tissue planting method and purified through transfer of single conidium to potato dextrose agar (PDA) medium (Kamal & Mai 2009). Maraitte et al., (1998) had also same observation while studying the 27 isolates of *Bipolaris sorokiniana* and found different colours of the colonies on minimal medium varied from white to light pink and dark green. The colony colors of 87 isolates of *B. sorokiniana* collected from different agro-ecological zones during 2004 and 2005 were observed. Four different types of colors of isolates were found on PDA medium (Asad, et al., 2009). Post-harvest diseases destroy 10-30% of the total yield of crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield (Agrios, 2005; Kader, 2002). Fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxins (Philips, 1984; Moss, 2002, Stinson et al., 1981).

*Alternaria* rot of citrus is a serious problem in citrus production worldwide. In Arizona, the disease is most commonly found in Minneola tangelos and navel oranges grown in Maricopa County In these studies, 218 fungal isolates were isolated from fruit lesions on tangelos, tangerines, lemons, oranges, and grape fruit from areas around phoenix & Yamq (Barrly et al., 2003). The isolates were identified as *Colletotrichum capsici, C. gloeosporioides* and *Alternaria alternata*, based on morphological and cultural characters.
Colletotrichum capsici was the most commonly isolated fungal species from infected chili fruits (Sriniyasan, 2010). Fungi isolated were identified as Alternaria alternata (from apple, bell pepper, bitter gourd, bottle gourd, papaya, pear, round gourd, sponge gourd and tomato), Alternaria citri (from sweet orange) and Geotrichum candidum (from apple, egg plant, carrot, cucumber, guava, melon, papaya, pumpkin, sponge gourd and turnip) Naureen, (2009). The fungi isolated from rotten apple fruits caused by Alternaria tenuis (Ilyas et al., 2007).

Six single sclerotium isolates of crown and root rot of Macrophomina phaseolina from strawberry (Aviles et al., 2008). On the basis of these characteristics, the isolated fungus was identified as M. Phaseolina (Wyllie, 1993; Watanabe, 2002). Seven isolates of Macrophomina phaseolina incitant of maize charcoal rot were established from major maize growing agro-ecological zones of India (Shekhar, 2006). Isolate of M. phaseolina isolated from infected chick pea (Khan, 2008). Isolation from infected tissue on PDA yielded a Macrophomina phaseolina growth with fluffy mycelium, fast growing, and grey in the beginning later on turning black in colour with abundant sclerotia of Tephrosia purpurea (L.) pres.(Majumdar, 2006).

The root rot pathogen Macrophomina phaseolina was isolated from greengram plants showing typical root rot symptoms, and pure cultures of the pathogen were obtained by the single hyphal tip method (Rangaswami, 1972). Sixty isolates of Macrophomina phaseolina, the cause of charcoal rot, were isolated from different parts of Iran on various plants including cantaloupe, long melon, soybean, cucumber, apricot, rosemary and sesame (Edraki and Banihashemi, 2010).

Sour rot, caused by the fungus Geotrichum candidum, Lk. ex Pers., is a decay of numerous fruits and vegetables, including citrus (Brown, 1979). The comprehensive survey of market storage and post harvest
fungal diseases of some important fruits in the market of Osmanabad district was undertaken by Bhale (2011). Sour rot disease incited by *Geotrichum candidum* causes serious damage to lime fruits during handling, transportation, marketing and exportation processes. Saprophytic yeast isolates were isolated from the surface tissues of various fruits and vegetables with high frequency in addition to two identified (Abd-Alla et al., 2007). Fungal cultures were isolated from *Geotrichum candidum* is naturally infected of tomato fruits (Sharma, 2006). *Geotrichum candidum* Link ex pers, is a fungal species which can infect and invade a wide range of hosts and substrates (Carmichael, 1957).

It has been isolated as the cause of sour rot in numerous horticultural commodities including citrus fruit (Smith 1917; Kuramoto and Yamada, 1975), tomatoes (Poole, 1922), cantaloupes (Wade and Morris, 1982), peaches (Wells, 1977), lychee (Jamaluddin, et al., 1975) and carrots (Wright, et al. 1964).

**Pathogenicity:**

Isolations of fruit rot of *Coccinia indica* by *Bipolaris tetramera* and pathogenicity test was conducted by Sharma (2006). On the other hand, pathogenicity of several *Bipolaris* spp. has been clearly established on bermudagrass. *Bipolaris* leaf spot caused by *Bipolaris cynodontis* (Marignoni) Shoemaker is considered to be of major importance; this species infects during cool wet weather and may cause extensive damage when it attacks crowns, stolons, or rhizomes. (Smiley et al., 2005; Couch, 1995).

The pathogenicity was proved by inoculating the injured healthy fruits with spore suspension of *Alternaria alternata* isolated from rotted fruits kinnow (*Citrus deliciosa*) (Sharma et al., 2008). Isolations of new post-harvest soft rot of *Coccinia indica* caused by the pathogen
Macrophomina phaseolina and pathogenicity of the organism was confirmed as Koch's postulates were fully satisfied symptoms were critically observed and recorded Sharma, (2007). To prove the pathogenicity of Macrophomina phaseolina, associated with seeds, apparently healthy surface sterilized cluster-bean (Cyamopsis tetragonoloba) seeds were rolled on 7d old culture (Jaiman and Jain, 2008). (Godara 1994), reported the pathogenicity test against post harvest pathogens of kinnow (Citrus deliciosa). The result of the pathogenicity tests showed that all the isolated fungi were pathogenic on tomato fruits, from visual observation under the microscope; the fungi reisolated were the same as those used for the pathogenicity test. The causal organism was isolated and identified as R. bataticola, Macrophomina phaseolina and its pathogenicity was confirmed by Santakumari, et al, (2002). Studies on Macrophomina phaseolina have investigated variations in morphology and pathogenicity among isolates from soybean, common bean and cluster bean (Purkayastha et al., 2006).

Also the wound created by the cork borer initiated spoilage as it formed opening for the easy penetration of the pathogens. This result on the pathogenicity of R. stolonifer, Fusarium moniliforme and Geotrichum candidum (Chuku et al., 2010). The isolated tomato fruits spoilage fungi from infected tissues included Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer and Geotrichum candidum. The results of the pathogenicity test showed that these rot fungi were able to cause deterioration after 7 days of inoculation (Yeni et al., 2010). The results showed the pathogenicity of isolates was different and themost aggressive isolates were from Mexico, Brezil and Colombia (Das et al., 2006). Finally we identified the six isolated yeasts as Geotrichum candidum in order to confirm host range and pathogenicity of the six newly-identified G.candidum isolates, ten vegetable crops including potato, sweet potato,
carrot, melon and oriental melon were inoculated with conidial suspension of each *G. candidum* isolates (Yong-Ki Kim et al., 2011). Tomato rot identified as *Fusarium moniliforme*, *Rhizopus stolonifer* and *Geotrichum candidum*. Pathogenicity test was carried out to determine if the organisms responsible for spoilage were host specific to tomato fruits, (Osakwe et al, 2010).

The pathogenicity test revealed that threen spoilage fungi (*F. oxysporum*, *Aspergillus niger* and *Aspergillus flavus*) induce rot in yams (Okigbo and Nwaka, 2005). Pathogenicity tests were performed with all three organisms, and we concluded that each of these yeasts by itself and in combination with one or both of the others was able to cause rot decay on stone fruit (Michailides et al., 2004).

**Sensitivity of the pathogens to different fungicides**

A potential new fungicide is identified in laboratory and glasshouse tests on different types of fungal pathogens, and is then tested in field trials against an appropriate range of crop diseases in different regions and countries. The pathogens it works against are deemed to be 'sensitive' and those that it does not affect or hardly affects are regarded as 'naturally' or 'inherently resistant'. This pre-existing type of resistance is of no further practical interest once it has been identified as a limitation to the range of use of the fungicide. Reasons for natural resistance are seldom investigated, although sometimes they can be deduced from mode of action studies. The 'fungicide resistance' we are considering here is a different phenomenon sometimes called 'acquired resistance'. Some people prefer to call this phenomenon 'insensitivity' or 'tolerance.' The former term is preferred by some plant pathologist, because they believe that fungicide resistance is easily confused with host-plant resistance to certain species or pathotypes of fungi.
The most common method is to grow the spore or any other propagule of pathogen on an agar medium containing different concentration of fungicide and find out minimum inhibitory concentration (MIC). The countries like U.S.A. and U.K have maximum cases of fungicide resistance in the world. In India attention towards fungicides resistance is focused by (Gangawane & Saler 1981; Annamalai and Lalitha Kumari, 1987; Chander and Thind, 1995). In the present review highlight are given of the aspects of carbendazim, resistance in Bipolaris tetramera (Mc kinney) and Alternaria plurisptata (Karst & Har) hard rot Macrophoma phaseolina Tassi (Goid) and Geotrichum candidus including their management of Laboratory and field resistance.

Sensitivity of different pathogens in relation to many fungicides has been reported (Jones and Ehret, 1976; Dekker & Gielink, 1979; Gangawane & Saler, 1981; Gangawane and Shaikh, 1988; Hollomon, 1981; Kamble, 1991 & Bhale, 2002). Annamalai and Lalithakumari (1996) suggested that it is essential to establish the base line sensitivity for the fungicide against sensitive strain, Brain (1980) considered that heterogeneous population of nuclei consisting of resistant and sensitive nuclei in the isolates might be responsible for variation in the MIC of fungicides.

Correlates with findings of (Ahmed et al., 2002) that non specific fungicides to Bipolaris oryzae are effective only at higher concentrations. (Sisterna and Ronco, 1994) reported Dithane M-45 as the best fungicide against Bipolaris oryzae, with the greatest effect at 500 ppm. In this concentration, all the fungicides significantly reduced the mycelial growth. However, Tilt 250 EC, Bavistin and Hinosan also showed inhibitory effect on the growth of Bipolaris oryzae. Viswanathan and Narayanasamy (1992) reported significant result with the same fungicide at 1000 and 2000 ppm the lowest effect of Bavistin and Hinosan and also
supported by Swaminathan and Lalithakumari (1982). Geetha and Sivaprakasam (1993) reported that Bavistin carbendazim was effective against **Bipolaris oryzae**, the causal agent of brown leaf spot of rice.

Cycloheximide, Cycloheximide-thiram, Chlorothalonil, Dyrene and Maneb are reported to be effective against different species of **Bipolaris** causings leaf spots or blight of turf grasses, the crown and roots (melting out) of turf grasses (Agrios, 1988).

Copper fungicides are widely used for **Alternaria** control in Florida (Timmer, 2003) and when applied on a timely basis provide good control of the disease. Numerous products that induce resistance in the host have been evaluated for control of **Alternaria** brown spot (Agostini et al., 2003). Fruit rot caused by *A. alternata* and the pathogen was effectively controlled by dipping the fruits for 10 min in Carbendazim solution of 1000 μg/ml concentration (Datar, 1996). Fugro et al. (1994) evaluated some fungicides for disease control and found that Dithane M-45 was significantly superior to others against *A. cucumerina* causing leaf blight of watermelon. Devanathan and Ramanujan (1995) reported that five fungicides (carbendazim, captafol, mancozeb, copper oxychloride and chlorothalonil) were applied on 2 tomato cultivars susceptible to infection by **Alternaria solani**. All the fungicides gave same disease control but Mancozeb gave the best control and highest crop yield followed by chlorothalonil and copper oxychloride. Sawant and Desai (2001) studied the efficacy of Dodine 65 WP and Mancozeb 75 WP with alachlor 50 EC and acephate 75 SP against early (**Alternaria solani**) on tomato.

Mancozeb has been reported to be effective fungicide against **Alternaria alternata** (Singh and Milne, 1974; Amaresh, 1997; Desai, 1998 and Sood and Sharma, 2002). Under *in vitro* studies mancozeb @ 2000 ppm was most effective in inhibiting the mycelia growth of **Alternaria tenuissima** leaf blight of **Chrysanthemum morifolium** Ramat
(Hegde, 1998). Arun Kumar (2008) reported that chlorothalonil and mancozeb were next to hexaconazole in terms of efficacy in controlling the leaf blight of chrysanthemum caused by *Alternaria alternata*.

Of the Six fungicides tested *in vitro*, mancozeb followed by captafol and copper-oxychloride was most effective in inhibiting mycelial growth and conidial germination of *Alternaria alternata* (Lalesh et al., 2006). Such as *Alternaria* blight of water melon (Bedi, et al., 1993; Fugro et al., 1994), sunflower (Singh 2000, Rao and Ran ganatha, 2003) and brinjal (Singh and Rai, 2003).

Ilyas et al. (1975) studied the efficacy of various fungicides and observed that carbendazim, quintozene and mancozeb reduced *Macrophomina phaseolina* population under laboratory conditions. Kulkarni (2000) screened various chemicals against safflower root rot caused by *Macrophomina phaseolina* and concluded that carbendazim and propiconazole were the most effective fungicides. Owing to less availability of investigation relating to sensitivity of different isolates to copper sulphate and carbendazim, review of work relating to other chemicals has been recorded in this section.

Some of the fungicides, which have been reported to be effective against *Macrophomina phaseolina* are captan, thiram and agallol (Grewal and Vir, 1958; Clinton, 1960; Bhargava, 1965; Sahai, 1969 and Masih, et al., 1970). Brassicol (Penta chloronitrobenzene) a soil fungicide has been reported to be effective against *M. phaseolina* isolated from various crops other than sorghum (Goel and Mehrotra, 1973). Further, it has also been reported that. *M. phaseolina* has developed resistance against increasing concentrations of brassicol (Grover and Chopra, 1970; Mathur and Singh, 1973), other workers also reported that, brassicol, did not control the disease in field trials (conducted between 1976-78) and in *in vitro* evaluation on PDA, it inhibited the growth upto 86.75 per cent at 5000
ppm, but in soil at the same concentration, inhibition was only up to 15.25 percent (Anahosur and Patil, 1983). Rajkule et al. (1979) reported that soil treatment with thiram at sowing, did not effectively control charcoal rot, but reduced it by 25 percent.

Anahosur et al. (1984) found that an herbicide called ametryne inhibited the growth of the pathogen (28.5 to 60.15% at 250 to 2000 ppm). Sekhar (1985) observed that rhizolex followed by quintozene were significantly superior in suppressing saprophyte activity of *Macrophomina phaseolina*. Hundekar (1987) reported that carbendazim and rhizolex were effective against *Macrophomina phaseolina* and thiram was effective among non-systemic fungicides. Pall et al., (1990) opined that seed treatment with MBC (carbendazim) followed by thiram were the best fungicides for controlling *Macrophomina phaseolina* in urd and green gram. Gupta and Ray (1993) opined that S-methyl-S, diphenyl phosphorotrithioate was most active *R. solani*, while its P-methyl analogue was more effective against *R. bataticola* (*M. phaseolina*).

Ramdoss and Sivaprakasan (1987) reported inhibitory effect of carbendazim and quintozene, carbendazim at 100 ppm and thiram at 500 ppm were fungicidal, while quintozene and all insecticides were fungistaic. Singh and Kaiser (1995) reported that among the eight fungicides tested under *in vitro* and field conditions, carbendazim was found most effective against *M. phaseolina*.

Researchers also have been investigated the *in vitro* Sensitivity of different isolates of *M. phaseolina* to fungicides (Al-Beldawi et al., 1973). The efficacy of fungicide application to seed and soil to reduce fungal germination and infection (Kannaiyan et al., 1980; Alice et al., 1996). However, until now chemical control of *M. phaseolina* is difficult and neither profitable nor advisable (Pearson et al., 1984). Bavistin 50 WP (carbendazim) was the most effective against *M. phaseolina*, a major
pathogen of mothbean (Rathore and Rathore, 1999). Application of carbendazim in combination with thiram resulted in the highest seed germination percentage and lowest root rot incidence in chickpea (Prajapati et al., 2003). Dubey (1991) reported that *Macrophomina phaseolina* was inhibited by carbendazim and mancozeb. Chaudhary and Sharma (1988) reported increased inhibition of *M. phaseolina* with increased concentration of 14 fungicides tested. Thakare and Gahukar (1999) reported that carbendazim and mancozeb completely inhibited the growth of *Macrophomina phaseolina* causing leaf and stem blight of greengram.

Effect of BA and SA and their salts Sb and Ps as well as chitosan and the fungicide sodium ortho-phenyl-phenate (SOPP) at different concentrations, i.e., 0 (Control), 0.5, 1.0, 2.0 and 4.0%, were tested for their inhibitory effect on linear growth of *Geotrichum candidum* (Abdel, 2011). The tolerance of *G. candidum* to respiration fungicides is not surprising because this yeast is known to be tolerant to other site-specific fungicides (Kaul et al., 1977). The measures employed to manage postharvest citrus rot are not effective against *Geotrichum candidum*. This pathogen is not controlled by any of the fungicides (e.g., Imazalil and Thia bendazole) registered for use on citrus fruit (Eckert, 1978; Feng et al., 2011; Liu et al. 2009; Smilanick et al., 2008, Mercier and Smilanick, 2005, Brown, 1988). Benzimidazole fungicides (Benomyl, Carbendazim and Thiabendazole) have been found effective as fruit dip for control of postharvest decay of guava fruits (Arya et al., 1981; Bhargava and Singh, 1974; Gupta et al., 1973; Majumdar and Pathak, 1997). During storage conditions sapota gets severly infected by *Geotrichum candidum* causing sour rot (Mickelbart, 1996). In present paper three fungicides viz- Carbendazim (50% WP), Mancozeb (75% WP), and Captan (50% WP) were tested at different concentrations against *Geotrichum candidum*
causative fungi on sapota for determination of their effects on the radial growth of causative fungi, (Wagh and Bhole 2012).

**Physiological and biochemical changes of the pathogens**

**Physiological characteristics**

The sensitivity of *Bipolaris Drechslera* and *Exserohilum* to 11 different fungicides and 9 kinds of carbon sources were tested *in vitro*, (Yamaguchi and Mutsunobu, 2010). This phenomenon is known as genetic vulnerability and its biochemical and physiological base is the consequence of the changes the occur in genes and enzymes (Van Der Plank, 1975). Many authors (Milner et al., 1996; Czaczek et al., 2000; Nielsen et al., 1999) obtained a much better result with more natural carbon sources.

Bhandari and Singh (1976) observed that fructose and mannose supported better growth of *Alternaria triticina* compared to ribose, arabinose, xylose, sucrose, glucose, maltose and lactose. Goyal (1977) found that growth of *Alternaria alternata* was maximum on maltose followed by sucrose, starch, glucose and maltose and poor growth was noticed on galactose and mannitol. Rane and Patel (1956) found ammonium nitrate, potassium nitrate, sodium nitrate and peptone as the best nitrogen sources for the growth of *Alternaria macrospora*. Hasija (1970) reported that *A. citri* and *Alternaria alternata* metabolised three major sources of nitrogen *viz.*, nitrate, ammonical and organic form of nitrogen. Fungi require nitrogen in same form or other, however preferential source vary in different fungal types (Dansentos, 1963). Padmanabhan and Narayanaswamy (1977) reported that sodium nitrate and urea supported good growth of *Alternaria macrospora* among various inorganic and organic nitrogen sources tested. Mohapatra et al. (1977) observed that ammonical form of nitrogen was superior to nitrate form
and ammonium chloride was the best followed by ammonium oxalate in *Alternaria sesame* in sesame host.

Patil and Kulkarni (1965) reported that, arabinose, dextrin, glucose, lactose and sucrose supported good growth of *Macrophomina phaseolina* isolated from cotton, sesame, groundnut and castor. Moniz and Bhide (1963) working with the Gujarat strain of *Macrophomina phaseolina* observed that, dextrose, sucrose, raffinose, levulose and maltose supported good growth and sclerotal formation of the fungus. Shanmugam and Govindaswamy (1973) obtained significantly higher growth of *Macrophomina phaseolina* in asparagine followed by glutamine, peptone and potassium nitrate. *Raiococtonia bataticola* metabolized a number of nitrogen compounds for the growth and that the amount of growth varied with the type of nitrogen source. Increased production of amino acid in mercury and capton resistant isolates of *Macrophomina phaseolina* have been noted by Rana and Sengupta (1976).

When granules were cultivated with acetate or glucose as a carbon source and nitrate as a nitrogen source, the identified bacterial strains were the members of the genus *Epistylis*, *Potieriochromonas*, *Geotrichum klebahnii* (Williams and Reyes, 2006). 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 case in hydrolysate, and from amino acids and vitamins tested, asparagin and biotin supported maximum growth and pycnidial production of *Phomopsis theae* (Ponmurugan and Baby, 2007).

Akinyosoye et al. (2003) had earlier reported that starch supported the maximum biomass yield of *Geotrichum candidum* and *phoma*
sorghina better than disaccharides (maltose and lactose), monosaccharides (glucose, fructose and galactose). Hussain et al. (2003) reported that, among all carbon sources starch was the best for growth of the sclerotium rolfsii whereas other carbon sources did not showed good results. Lee et al. (2000) investigated the dye-decolourizing peroxidase by cultivating Geotrichum candidum Dec1 using molasses as a carbon source.

In decolorization of melanoidin by Coriolus sp.No.29, the effective sugars were glucose and sorbose (Watanabe et al., 1982) Zhang et al., (1999) studied different carbon sources as effective co-substrates on decolorization of cotton belaching effluent by on unidentified white rot fungus and reported that glucose, starch; maltose and cellobiose were good carbon sources while sucrose, lactose, xylon, xylose, methanol and glyoxol were poor carbon sources. Chavan et al. (2006) worked on the microbial degradation of melanoidins in distillery spent wash by Pseudomonas sp. and reported that addition of externally added carbon source was essential for decolorization. Growth of Geotrichum candidum on different carbon sources with asparagin as a nitrogen source and on different amino acids with glucose as a carbon source have been reported (Duran et al., 1973).

Dinesha (1984) also found that Sucrose was the best carbon source for the growth of Cercospora sorghi. Khandar et al. (1985) opined that glucose supported the best growth of Cercospora canescens Ell. and Mart. followed by sucrose, maltose and fructose.

Biochemical characteristics

Naik et al. (1981) observed more phenols in healthy leaves of sorghum than in the leaves infected with rust. However, the quantitative and qualitative estimation of total phenols and orthohydroxy phenols determined in diseased and healthy leaves of different sorghum lines of
resistant, moderately resistant and highly susceptible to Helminthosporium rostratum. Ram Dayal and Joshi (1968) studied the post infection changes in sugar content of the leaf caused by Helminthosporium sativum (Sacc.) Subram and Jain, In barley and they observed that there was decrease in reducing, non reducing and total sugars of infected leaves than that of healthy leaves.

Bhatia et al. (1972) concluded that ability of tomato plants to resist infection by Alternaria solani depend on the quality of phenolics in the leaf, stem and roots of the plants. Higher amounts of total phenolics were found in the resistant variety than in susceptible variety. Malli et al. (2000) studied the effect of development of mungbean yellow mosaic virus on biochemical constituents of mothbean genotypes. Biochemical changes in the mycelia mat of Alternaria alternata and resistant to carbendazim compared with all parameters were found to be more in resistant isolate than sensitive one (Bhale et al., 2009).

Anahosur et al. (1985) found that higher levels of sugars and phenolics were present in sorghum genotypes resistant sorghum to Macrophomina phaseolina than in susceptible ones. Sharma et al., (1992) studied the biochemical relationship in resistant and susceptible cultivars of maize with Turcium leaf blight disease.

Rhizopus stolonifer (Rhizopus rot), Fusarium roseum (Fusarial rot), Gleosporium musarum (Anthracnose) and Aspergillus niger (Aspergiulls rot) were more common causing considerable damage. Four fungi were studied from Banana fruits which cause considerable biochemical changes to alter the quality of banana fruit the infected bananas showed a decrease in the quantity of total soluble sugar, protein, ash, ascorbic acid and mineral elements when compared with control of fruit (Sawant and Gawai, 2011). Siddaramaiah and Hegde (1990) found that Cercospora infection induces changes in the biochemical constituents
like total amino acids, phenols and sugar which may affect the quality of mulberry leaves.

Physiological and biochemical characteristics of the Geotrichum candidum strain. It was found that the strain fermented sugar such as glucose and galactose, and was resistant to cycloheximide. (Loo et al., 2006). Garcia et al. (2000) used the species Aspergillus niger, Aspergillus terreus and Geotrichum candidum in the removal of phenolic compounds. Similarly, Setty et al. (2001) found that the resistant cultivars of maize had significantly higher amounts of sugar against P. sorghi infection. Khillare et al. (2005) reported total sugars, total amino acids, and crude protein DNA and RNA contents increased in their quantity due to infection by both the isolates of fruit rot of grape.

There are many workers who have also studied physiological and biochemical characteristic of fungal pathogens (Bharade, 2002; Wadikar, 2002; Hiwale, 2003; Telmore, 2004 and Patil, 2009). The ascorbic acid in the sample was calculated taking ascorbic acid equivalent of the standard DCPIP solution (Indian Pharma copia, 1996).Polyphenol oxidase (PPO) activity was determined by measuring the initial rate of quinone formation as indicated by an increase in absorbance at 420 nm (Coseteng, and Lee, 1987).

Free and total phenols were extracted using the Vinson et al. (2001) method. For free phenols, 50% methanol/ water and for total phenols 1.2 M HCl in 50% methanol/water was used. The total and free phenolic contents of the extract were determined using the modified FC colorimetric method (Dewanto et al., 2002).

Reducing sugars was estimated by the Nelson-Somogyi (1944) method. Starch was estimated in the residue left after sugar extraction. Of the product, 0.1 gm of sulphated ash was dissolved in 5% HCl and used for determination of mineral elements through atomic absorption
photometry (Indrayan et al., 2005). Naik and Hiremath (1988) reported that the total sugar content decreased in *Colletotrichum gloeosporioides* infected betel vine leaf. Increase in free amino acids may be due to proteolysis of fruit proteins catalyzed by the fungal enzymes (Arya, 1993). Sundares et al., (1988) reported that diseases leaves are biochemically poor in nutritive value and indicated the reduction of moisture, protein and sugar contents.

Tomoto inoculated with *Geotrichum candidum* and *Aspergillus niger* showed decline in ascorbic acid content with days of incubation when compared with control fruit. The total soluble sugars of inoculated tomato fruit also showed a reduction trend as the storage period was prolonged (Oladiran and Lwu, 1992). Couture et al. (1971) reported that phenolic compound have long been correlated with resistance of plants to infection agents. The result of present study are in conformity with earlier report (Lily and Ramadasan, 1979). It appears that higher concentration of total phenols and ortho-dihydric phenols in resistant variety has offered protection to the plant from pathogen. Partial elimination of these phenolics compounds was obtained by using *Geotrichum candidum* (Borja et al., 1993). Similarly, the investigation carried out by Khodke and Wankhede (2000) revealed less content of ascorbic acid, total sugar and capsaicin in fruits heavily infected due to dieback. Patil and Kulkarni (1977) reported that the healthy leaves of sunflower contained more polyphenols than the leaves infected with *Puccinia helianthi* Schw. Borthakur and Addy (1988) observed that the phenol content in the leaves of the rice cultivars was reduced after the infection by sheath blight pathogen. Katrodia (1988) and Lime et al. (2001) also observed an appreciable reduction in reducing and non-reducing sugars in the damaged fruits.

**Synergistic effects of other agro-chemicals of the pathogens**
Most investigations aimed to study the effect of fungicides alone on the mycelial growth of *Alternaria solani* (Al-Malla, 1998; Brammall, 1993; Datar and Mayee, 1985 Jovancev, 1998). On the other hand, there are a few available reviews about the effect of mixing different types of pesticides, *i.e.* insecticides, fungicides, acaricides and nematicides on fungi growth especially *Alternaria solani*. Csinos et al. (1986) studied mixing of metalaxyl and fenamiphos through irrigation water to control black shank and root knot complex on tobacco fields. The results indicated that black shank and root knot complex was best managed where both chemicals were applied together. Hill and Stratton, (1991) reported that the fungicide chlorothalonil and herbicide metribuzin interacted in an additive manner at concentrations up to 900 ppm and antagonistically at levels greater than 900 ppm toward *Alternaria solani*. Atia, (1996) found that mixing fungicides Karathane, Rubigan, Afugan or Bayleton with the insecticide Actellic increased their efficacy against powdery mildew of cucumber except Byleton. Shaaban, (1993) reported that fungicides Antracol, Ridomil, Mancozeb, Sharis and Dithane M 45 were considered as only additive effect when mixed with the tested insecticides, except in case of Antracol / Sumicidin S.haris / Curacron, Dithane M-45 / Curacron and Dithane M-45/ Larvin gave.

Dubey (1991) reported effective inhibition of *Macrophomina phaseolina* by endosulfan and monocrotophos and further observed that a concentration of 500 to 1000 ug per g was needed to kill Sclerotia of *M. phaseolina*. Gangawane and Kamble (2001) found that when carbendazim was used in combination with agrochemicals inhibited the growth of resistant isolate of *Macrophomina phaseolina* causing charcoal rot of potato. Besides the *in vitro* analyses, Kataria et al. (1990) also investigated on disease control in crop plants and weed control.
Cyproconazole provided better disease control when it was combined with other herbicides, than applied alone.

According to Patil (2009) carbendazim in combination with fungicides (Captan, Zineb, and Mancozeb), insecticides (methomyl, endosulphan and monocrotophos) herbicides (2,4-D, Excel mera 71, and zepaclav 500), antibiotics (Griseofulvin and Ofloxacin 400), salt (potassium chloride and magnesium chloride), fertilizers (urea, muriate of potash, 10;26;26, mixture of urea, super phosphate, sufia, nimboli and potash), micronutrients (Mb, Co, Cu, Mn) completely inhibited the growth of *Macrophomina phaseolina* causing charcoal rot of sweet potato. Tilt in combination with fungicides (mancozeb, zineb, captan and rako), insecticides (Thimate and endosulphan), antibiotics (streptomycin and Griseofulvin) completely prevented the infection of *Phakopsora pachyrhizi* to soyabean plants, More (2009). Griffin (1981) reported that fungicides and antibiotics having different mode of action are able to control resistant pathogen.

According to Horsten (1979) mixture of benomyl and captan reduced the rate of infection to apple seedling by *Venturia inaequalis*. Further he observed that alternate treatment of carbendazim and naurimol completely prevented the infection of *Cercospora spp. in vitro* to the host. Heweyid et al. (2005) reported that copper oxychloride was the most inhibiting to Bradyrhizobial strains, even though it significantly decreased the infection percentage with *Macrophomina phaseolina*, *Fusarium oxysporum* and *Sclerotium rolfsii* compared to other tested fungicides.

The growth of some organisms has been inhibited by herbicides which are meant to destroy weeds while some fungi have been affected by application of insecticides. (Chen et al., 2001; Das et al., 2003). Streptomycin mixed with oxytetracycline is used in control of fire blight of pome fruits and could delay the appearance of resistant strains.
Application of carbendazim in combination with Thiram resulted in the highest seed germination percentage and lowest root rot incidence in chickpea (Prajapati et al., 2003).

Positive synergistic effects of combined chemical and hot water treatments have been corroborated on various fruit species (Wells and Harvey, 1970; Gutter, 1978; Sharma and Kaul, 1990; Barkai-Golan and Apelbaum, 1991; McDonald et al., 1991; Cohen et al., 1992; Coates et al., 1993; McGuire and Campbell, 1993; Schirra and Mulas, 1993; Conway et al., 1994; Rodov et al., 1994b; Schirra and Mulas, 1995b; Smilanick et al., 1995). Synergistic effect of combining biocontrol agents with silicon against postharvest diseases of jujube fruit (Shiping et al., 2005). Yeasts as biocontrol agents have been widely used to control postharvest diseases in various fruit because they do not produce antibiotics (Droby et al., 1998). 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).

Study of enzymes and toxins of the pathogens

Enzymes activity

Hydrolytic enzymes such as cellulase and pectinase etc., In case of post-harvest pathogens have been studied by some workers. These enzymes were found to be helpful during invasion and colonization by various plants pathogens. CMC proved highly inhibitory for amylase production where as glucose and fructose stimulated the amylase activity in Alternaria alternata, A. crassa, A. dianthicola and A. tenuissima. Lipase production was found to be stimulated in the presence of disaccharides and polysaccharides in all the species of Alternaria (Rathod and Chavan, 2010). Sreekantaiah et al. (1973) reported that Alternaria alternata, Fusarium solani, f. sp. minus, Pleospora infectoria, Alternaria solani. Were capable of producing pectinase and cellulose type of

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enzymes which results into post-harvest biodeterioration. Cellulose is commonly degraded by an enzyme called cellulose, produced by several microorganisms, commonly by bacteria and fungi (Bahkali, 1996; Mangelli and Forchiassin, 1999; Shin et al, 2000).

The strains have been mutagenized and genetically modified to obtain an organism capable of producing high levels of cellulases (Mandels and Andreotti, 1978; Nevalainen et al., 1980; Durand et al., 1988). Two mechanism of (Szengyel et al., 2000). Two mechanisms of glucan degradation have been reported; exo – and endo- glucanases, both of which act synergistically in glucan degradation β-glucan degrading enzymes are classified according to the type of β-glucosidic linkages, 1,4-β-glucanases (including cellulases), 1,3- β -glucanases and 1,6- β-glucanases (Pitson et al., 1993), Lytic enzymes, such as chitinases, 1,3- β- and β-1,6-glucanases, and proteases have been shown to play a critical role in the process of mycoparasitism (Chernin & Chet, 2002; Viterbo et al., 2002). Several individual hydrolytic enzymes have been overexpressed in T. virens and other Trichoderma spp. often resulting in enhanced biocontrol activity.

Cellulose has enormous potential as a renewable source of enzyme (Coral et al., 2002). A great variety of fungi and bacteria can fragment these macromolecules by using hydrolytic, enzymes and used as a carbon source (Acharya et al., 2008). Higher values of enzyme activities for beta glucosidase was obtained from sample in which corn steep liquor was used as a Nitrogen source as compared to control (Siddique et al., 2010). Okunowo et al., (2010), reported that carboxym ethyl cellulose (CMC) was the best carbon source for maximum enzyme production in Carvularia pallescens showing b-glucosidase (0.99µ/ml) and CMCase (0.95µ/ml) activity.
Whitaker, (1991), reported that pectinase enzymes are produced by number of bacteria, yeast fungi, protozoa, insect, nematode in order to degrade (to obtain carbon source) or to modify in (Fruit ripening ) the hertopoly saccharide pectin. Several workers worked on the production of pectinase in the hydrolysis of carbohydrates due to post-harvest fungi. However pectinase production in post-harvest fungi was directly related with reduction in quality of fruits. Pandey and Gupta (1966) found that presence of glucose in any concentration was inhibitory to enzyme secretion by Alternaria tenuis, Akhter et al, (2011), reported that pectinase activity was higher in wheat bran and potato starch media, similar findings were also reported by Fujio and Eledago, (1993) in Rhizopus spp. Several workers reported the role of toxic metabolites of storage fungi on seed germination. Soyabean seed showed reduction in germination percentage, which were soaked in filtrate of phomopsis phaseoli (Hilty and Lee, 1988).

A numbers of cell wall degrading enzymes have been shown to be produced by plant pathogens (Chenglin et al., 1996), which are known to facilitate cell wall penetration and tissue maceration in host Plants. Chandler and Klostermeyer (1983) studied the production of lipase by Geotrichum candidum under various growth conditions.

**Toxins activity**

Seed treated with *M. phaseolina* reduced seed germination. *M. phaseolina* produce toxin known as phaseolinon which inhibit seed germination up to 50% (Bhattacharya et al., 1994) Soyabeans seeds soaked in culture filtrates of fusarium solani, F. oxysporum, Aspergillus niger, A.flavus, Alternaria tenuis and A. alternata, for 24 hours showed reduction in percentage seed germination (Ibrahim et al., 1987). The inhibitory nature of culture filtrate produced by seed borne fungi in
different crops have been reported as *Curvularia lunata* found to be active toxin producer among Jowar seed mycoflora (Bhale et al., 1982).

Both the toxins caused wilting in several plants, namely bean, brinjal, rice, tomato and wheat, showing their non-specific nature, (Rai, 1977). Most of plant bacteria toxins studied are non- specific (Kuo et al., 1970; Patil, 1974; Rai, 1976; Stroble, 1974). The synergistic effect with enzymes produced by them also appears to be important (Belanger et al., 1995; Shirmbock et al; 1994). It is used for seed treatment and controlled *Rhynchosporium secalis* in spring barley (Kulisler, 1997).

Phytotoxin refers to a substance produced by a plant that is toxic or a substance that is toxic to the plant. Many substances produced by plants are secondary metabolites and are the byproducts of primary physiology processes. Some examples of phytotoxins are alkaloids, terpenes, phenolics, herbicides and substances produced by microorganisms. Shanmugum et al., (2001) was made purification and characterization of phytotoxin alpha-glucosidase protein from *Trichoderma viride*.

Sriram et al., (2000) and these isolates reduced the severity of toxin- induced symptoms caused by *Rhizoctonia solani* on rice.

In addition, several toxins produced by microorganisms were reported to be responsible for the induction of diseases in planst. These microorganisms produce toxic metabolites in culture media and plant tissues which were involved in the disease syndrome (Wood et al., 1972). Several species of *Colletotrichum* and *Alternaria* were known to produce different types of toxic metabolites (Bhaskaran and Kandaswamy 1978; Sriram et al., 2000).

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). Reduction in seed germination and radical growth of coriander due to the harmful effect of secondary metabolites of *Fusarium* (Hashim and Thrane, 1990). 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 Varticiillum alboatrum was found to inhibit cell growth and reduced the viability of alfalfa seed (Frame et al. 1991). Abraham (1978) was also reported the inhibitory effect of culture filtrates of fungi on seed germination.

**Eco-friendly approaches for the mangement of the pathogens**

Today there are strict regulations on chemical pesticide use and there is political pressure to remove the most hazardous chemicals from market. Thus alternative approaches are preferred which are ecofriendly (Anadraj and Leela, 1996). Therefore it is important to find practical, economic and non-toxic methods to prevent fungal deterioration of fruits. Biological control of phytopathogen is ecofriendly and cost effective hence it should become an important component of plant disease management practices. There is an enormous amount of literature on the pre and post harvest pathology of fruits, their management. Some relevant important literature by Indian workers is by Das Gupta and Mandal (1988); Pathak (1997); Pathak et al. (2001); Sharma and Alam (1998).

The price of fruit is due to its flavour, nutritional value and taste. Being soft textured *Coccinia indica* fruits are highly sensitive to exogenous agencies specially fungi that affects physiology, morphology and biochemistry of fruits and thus ultimately causes loss to the fruit seller. The fungal plant diseases are usually controlled by application of
fungicides (Maloy, 1993). However extensive use of chemicals as antifungal agents might lead to severe side effects such as carcinogenicity, teratogenicity, oncogenicity and other genotoxic properties (Basilico and Basilico, 1999). and further extensive use of chemicals leads to biohazardous effects on ecosystem.

Secondary metabolites are antimicrobial in nature and are produced by plant 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 effectivenees as antifungal agents for possible use against pathogenic fungi. Therefore, the present investigation was undertaken to evaluate different native bioagents (Trichoderma spp), Plant leaf extract, Plant latex, and essential oils under in vitro conditions.

**Plant extract**

Control of anthracnose fruit rot, has for many years relied on chemical control and resulted in many undesirable problems, environmental pollution, accumulation of toxic substances and development of resistance in plant pathogens. Akhter et al. (2006) was tested eight ethanolic plant extracts and ten aqueous plant extracts in combination with cow urine to inhibition of conidial germination of Bipolaris sorokiniana causing leaf blight disease of wheat and recorded that Adhatoda vasia (leaf) and Zingiber officinalis (rhizome) extracts were most effective in inhibition of conidial germination at 2.5% concentration where, most cases Ocimum sactum extracts exhibited less inhibitory effect. Shekhawat and Prasad (1971) reported that out of nine plant extracts tested viz., Allium cepa L., Allium sativum L., Ocimum sanctum L., Mentha piperita L. and Beta vulgaris L.showed strong inhibitory action against Alternaria tenuis Nees. From bean,
*Helminthosporium* sp. from watermelon and *Curvularia pennisetii* (Mitra) Boed. From bajra used as test fungi.

Nargis et al. (2006) reported that the extracts of *Adhatoda vasica* Nees., *Zingiber officinale* L., *Vinca rosea* and *Azadirachta indica* Juss. In combination with cow dung, *Calotropis procera* (Aiton) W.T. Aiton and cow urine posses high ability to inhibit conidial germination of *Bipolaris sorokiniana* which might be used for controlling phytopathogens of crop plants. According to Khan et al. (1998) aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium tarsicum* and *Ascochyta rabiei* and that of *Calotropisprocera* against *Alternaria redicinua*.

Antifungal activity of crude extracts of plants, Hussein et al. (2002) reported that leaf extracts of *Datura stramoniam* reduced the development of rust pustules on the leaves of wheat. Mughal et al. (1996) observed that aqueous leaf extracts of *Allium sativum*, *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternate*, *A. brassicola* and *Myrothecium roridum*. Aqueous extracts from all the plant species tested, significantly reduced conidial germination of *Alternaria porri*. Maximum reduction was observed with a leaf extract of *Polyalthia longifolia*, followed by *Eucalyptus citriodora*, *Datura alba*, *Ocimum sanctum*, *Punica granatum*, *Azadirachta indica*, *Ipomoea carnea*, *Tridax procumbens* and *Tabernamontana coronaria* (Datar, 1994). Pawar and Kolhe, (2010) reported the botanicals like leaf extract, rhizome and seed extract of different plants. Ten % aqueous leaf extract of *Parthenium hystrophorus*, *Azadirachta indica*, *Adhatoda vasica* and *Aegle marmelos* retarded the growth of *Alternaria alternata*.

Hannan et al. (2005) used various plant extracts on seeds to investigate the development of black point infection due to *Alternaria alternata* in wheat grains. Henna (*Lawsonia inermis* L.), Jimson weed
(Datura stramonium L.) and Neem (Azadirchta indica A. Juss) were the most effective and suitable for the control of Alternaria alternata, one of the causal organisms of black point disease.

Bajwa et al. (2005) studied in vitro antifungal activity of aqueous leaf extracts of two Eucalyptus spp. viz., E. camaldulensis Dehnk and E. citriodora Hook, against three pathogenic fungi, namely, Alternaria alternata, Drechslera hawaiensis and D. tetramera to screen effective natural substances as an alternative to chemical fungicides.

Dubey et al. (2009) also found that different extracts of neem plant parts including leaf, bark, oil cake and neem oil against mycelial growth of Macrophomina phaseolina isolated from charcoal rot of soybean. The efficacy of the other leaf extracts, namely, Acacia arabica, A. cepa, A. sativum has also been reported to be able to stop mycelia growth of Macrophomina phaseolina completely even at 5% (Dubey & Dwivedi, 1991). The aqueous and organic solvents (water and ethanol) extracts from leaves of Azardirachta indica Adr. Juss (Meliaceae) and Chromoolaena odorata (Asteraceae) where tested against fungal pathogens of rotten tomato (Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer and Geotrichum candidum) by poisoned food method (Yeni et al., 2010). (Talibi et al., 2012). The powders and aqueous extracts of 43 plant species, harvested in different regions of southern Morocco, were screened for their in vitro and in vivo antifungal activity against Geotrichum candidum, the cause of citrus sour rot.

Plant latex

The demonstration of antifungal activity of C. gigantean latex extract against fungal species may be an indicative of the presence of broad spectrum antibiotic compounds, (Larhsini, et al., 1999). Finally the samples were centrifuged as previously described & the clear soluble supernatant was collected & lyophilized. The stock solutions of latex
extract was diluted suitably as required from stock solution (Juncker et al., 2009). The fresh latex of *J. curcus*, *C. gigentea*, *F. bengalensis* and *F. glomerata* were aseptically collected from the aerial parts of the healthy plants as described by Aworh et al. (1994) in clean glass tubes containing distilled water to yield a dilution rate of 5:5 (v/v). Leaf extracts, chopped leaves and latex of *C. procura* have shown great promise as a nematicide *in vitro* and *in vivo* (Khristova and Tissot, 1995). The mycelial growth, percentage spores germination and germ - tube extension in *Fusarium oxysporum* and *Aspergillus carbonarius* decreased when *Calotropis procura* extract concentration increases, where as growth of *Humicola brevis* and *Penicillium lanosum* were not affected (Rizk, 2008).

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). Raghavendra (2011) reported the latex extract were screened *in vitro* against human pathogenic strains such as Gram positive; *Staphylococcus aureus, Bacillus subtilis*, Gram negative; *Salmonella typhi, Klebsiella phoenomenia* and two fungal stains; *Aspergillus niger* and *Candida albicans*. The inhibitory effect was assessed by agar well diffusion method.

**Essential oils**

The general antifungal activity of essential oils is well documented (Reuveni et al., 1984; Deans and Ritchie 1987; Alankararaao et al., 1991; Baruah et al., 1996; Gogo et al., 1997; Pitarokili et al., 1999; Meepagala et al., 2002) and there have been some studies on the effects of essential oils on post-harvest pathogens (Bishop and Thornton, 1997).

Examined the *in vitro* effect of extracts of different neem (*Azardirachta indica* A. Juss) plant parts such as leaf, bark, oil cake and
neem oil on the growth, mycelia yield and sclerotal survival of *Macrophomina phaseolina* (Dubey et al., 2009). Abdel-Kader et al. (2011) reported that carnation, caraway, thyme, peppermint and geranium essential oils have been found to have been found to have inhibitory effects against the mycelial growth of *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* under *in vitro* conditions.

Batra and Mehta, (1985) isolated essential oil from seeds of *Argyeria speciosa* and tested against *Geotrichum candidum, Alternaria solani, Helminthosporium* spp. and *Colletotrichum dematum*.

**Trichoderma species**

*Trichoderma viride* T112 and *T. viride* (MO), *T.harzianum* (M) and *T. harzianum* T194 were used as potential biological agent for control of common root rot caused by *Bipolaris sorokiniana* (Salehpour, et al., 2005). Kumar et al. (2007), reported *T. viride* as the most effective antagonist for *A. alternata* while Sempere and Santamaria (2007) found *T. harzianum* as the potential antagonist for *A. alternata*.

Potphode (2004) studied the efficacy of *T. viride* and *T. harzianum* against *C. gloeosporioides* causing anthracnose of Jasmine and found that maximum inhibition of the pathogen was achieved by *T. harzianum* when placed at the center of the test fungus. Kumar, (2006) reported strong antagonistic effect of *B. subtilis, A. niger* and *T. Viride* against *M. phaseolina in vitro* whereas, *A. flavus, T. harzianum, T. longibrachyatum, G. virens* and *P. fluorescens* appeared as potent antagonists.

The efficacy of antagonists in the control of *M. phaseolina* has been reported earlier in sesamum (Sankar & Jeyarajan, 1996), pigeonpea (Lokesha & Benagi, 2007), eggplant (Ramezani, 2008), and sunflower (Shahnaz Dawar et al., 2008). Different species of *Trichoderma* gained considerable success against pathogenic fungi. *T. harzianum* protects the
root system against *F. solani*, *R. solani* and *M. phasaelina* infection on a number of crops (Malik & Dawar, 2003). Sankar and Sharma, (2001) stated that 2 out of 9 isolates of *T. viride* evaluated in preliminary tests showed superior performance against *Macrophomina phaseolina* (the causal of charcoal rot in maize) in the laboratory. The efficacy of four fungal bioagents viz., *Trichoderma harzianum*, *T. polysporum* and *T. viride* were evaluated *in vitro* condition against the Eggplant root rot pathogen, *Macrophomina phaseolina* (Hesamedin Ramezani, 2008).

Swami and Mukadam (2004) observerd the efficacy the efficacy of *T. viride* aginst the tomato fungi *Geotrichum candidum*, *Aspergillus niger*, *Alternaria solani*, *fusarium oxysporum* and *Rhizopus stolonifer*. 