Fruits constitute an important item of our food and they play a vital role in the human diet through the supply of vitamins and minerals. The recommended quantity of fruits to be consumed by a normal healthy adult is 230 g/day while the present per capita consumption of fruits is reported to be below 160 g/day (Veeraragavathatham et al., 1996). Among the various fruits grown in India, mango is one of the most important tropical fruit and is called as the ‘king of fruits’. India ranks first in the world production of mango with a share of 54.3 per cent. Post harvest losses in fruits are due to many factors, among which post harvest disease is considered to be a principal cause. Dasgupta and Mandal (1989) described more than 300 parasitic diseases of fruits and vegetables after harvest. Mango is the worst sufferer based on the percentage loss over the marketable period (Mandal and Dasgupta, 1981). The estimated post harvest losses during 1987-88 varied from 17.1 to 36.7 per cent (Anon., 1990).

Studies on post harvest losses of perishables are limited and fragmentary. Losses due to diseases that occur in fruit during transit, storage and marketing are not less than those of field diseases (Chandra, 1986). According to Smith et al. (1964) there are more than 250 known parasitic diseases of fruit that cause decay and blemishes during transit, storage and marketing. The damage and losses incurred vary with crop, handling during harvest and storage and transit. Fruit losses occur in the orchard as well as during total post harvest systems of grading, packing, transportation, storage and marketing. The losses are immense in perishable fruits like mango than other crops (Sharma et al., 1994). On an average 17.7 % mangoes are decayed due to fungal diseases. Considering the commercial importance and extent of losses caused by storage rot, natural injuries rot,
transport injury rot and mechanical injury rot in mango, it was thought to identify the fungi associated with post-harvest spoilage.

**Post-harvest fungal Diseases of mango fruits**

Post- harvest diseases of fruits represent one of the most severe causes of loss of production (Eckert, 1977; Derbyshrine and Shipway, 1978; Harvey, 1978 and Coursey, 1983). The high moisture content of mango fruits make them highly susceptible to the attack of pathogens. Major loss of harvested mango fruits is caused due to fungi. The disease caused during pre harvest of fruit also responsible for the lowering the quality and market value of the fruit. Post spoilage of fruits may be due susceptibility of fruits to the fungal growth. Susceptibility of fruits to the attack of fungi is varying according to season, cultivar, mechanical injuries and handling at the time of harvesting as well as by post-harvest treatments and storage conditions (Derbyshrine and Shipway, 1978 and Dennis, 1983). The soft rind fruits are more susceptible than tough rind fruit. The injured fruits are highly susceptible than uninjured fruit ones in all types of fruits.

The most important post-harvest diseases of mango fruits are anthracnose, stem end rot, *Aspergillus niger* rot, soft rot, *Alternaria* rot (Arya, 1993 and Philip, 2002). Sangchote (1987) reported that *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Dothiorella dominicana*, *D. mangifferae*, *Phomopsis mangiferae* and *Aspergillus niger* are common post-harvest pathogens of mangoes in Thailand. Species belonging to *Rhizopus*, *Aspergillus*, *Colletotrichum*, *Botryodiplodia*, *Phomopsis* and *Diplodia* have been reported to cause rotting of fruits during transit, storage and marketing injuries (Dasgupta and Bhatt, 1946, Dasgupta et al., 1950, Verma and Kamal, 1951, Kanitkar and Uppal, 1939; Thakur and Chenulu, 1970; Laxminarayan and Reddy, 1975; Thakur, 1972). Fungal pathogens involved in mango rotting after harvest include *Colletotrichum gloeosporioides* responsible for mango anthracnose, *Alternaria alternata* and *A. tenuissima* that cause alternariose, *Botryodiplodia theobromae* and
**Dothiorella spp.** responsible for stem end rot and *Phoma mangiferae* (Dodd et al., 1997; Arauz, 2000).

The high moisture content of mango fruits make them highly susceptible to attack of pathogens. Fungal infections alone were causing 17 percent losses annually in Warangal. Lele et al., (1975) and Pathak (1980) reported that many fungal pathogens are attacking the mango fruits and majority of them are wound pathogens and causing extensive damage. Similar loss of harvested mango crop in India has been estimated to be about 40% by Singh (1960). The extent of loss caused by the individual pathogens has also seldom been estimated. Srivastava et al. (1965) while surveying the markets situated at different localities in India, have recorded that the post-harvest loss in mango varieties due to *Aspergillus* rot is 4-35%, *Colletotrichum* rot 5-15%, and *Botryodiplodia* rot 6.5-20%.

**Anthracnose:**

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., is the major postharvest disease of mango in all mango producing areas of the world (Dodd et al., 1997). The disease occurs as quiescent infections on immature fruit and the damage it incites is more important in the postharvest period (Muirhead and Gratitude, 1986 and Dodd et al., 1997). Anthracnose is the most important disease of mango in humid production areas (Arauz, 2000; Dodd et al., 1997; Lim and Khoo, 1985; Ploetz and Freeman, 2009; Ploetz and Prakash, 1997; Ploetz, 2003). In India, twenty different genera of fungi are known to attack a mango fruit during postharvest handling (Pathak, 1980) of which anthracnose caused by *C. gloeosporioides* is the most important (Snowdon 1990; Johnson and Coates 1993). Anthracnose is an important disease which causes loss in all over the world (Philip, 2002). In storage it is sever. Arya (1993) also reported that in storage anthracnose causes severe rotting of mango fruits.

It is reported that anthracnose cause heavy losses in mango to the extent of 15 percent in India (Tandon, 1967), 29 per cent in Bangalore (Sohi et al, 1973), 11 per cent in West Bengal (Mandal, 1981), 24 per cent in
Lucknow (Omprakash and Raoof, 1988) and 29.6 per cent in Himachal Pradesh (Sharma et al., 1993). At retail and consumer levels, the mango fruits were in the stage of ripening or fully ripe, since, the symptoms of the diseases on unripe mango manifested only upon ripening. Diseases such as anthracnose caused by *C. gloeosporioides* (Daquioug and Quimo, 1979; Sangchote and Chayasombat, 1986; Chandra and Pathak, 1992) and stem end rot caused by *B. theobromae* (Pathak and Srivastava, 1967; Palaniswami, 1978; Chandra and Pathak, 1989) showed latent infections.

According to Sangchote (1987) anthracnose first appeared as small black colour circular spots and later became sunken and collapsed forming lager spot. Entire fruit covered with dark blemishes in severe condition where as Arya (1993) observed anthracnose symptoms include sunken, blackish brown blotches upon which salmon buff masses of spore develop. Arauz (2000) reported that postharvest anthracnose shows symptoms in the form of rounded brown to black lesions with an indefinite border on the fruit surface. Lesions larger than 2 cm are fairly common. Lesions of different sizes can coalesce and cover extensive areas of the fruit, typically in a tear-stain pattern developing from the basal toward the distal end of the fruit. Lesions are usually restricted to the peel, but in severe cases the fungus can invade the pulp. In advanced stages of the disease, the fungus produces acervuli, and abundant orange to salmon pink masses of conidia appear on the lesions. Anthracnose symptoms are appear in the form of numerous brownish irregular spots located anywhere on the fruit surface. The spot become sunken and coalesce together to form large dull brownish areas on the rind and pulp become discoloured (Philip, 2002).

**Stem end rot:**

Stem end rot is caused by *Botryodiplodia theobromae* was reported to cause decay to the maximum extent of 20 per cent in India (Tandon, 1967), 50 per cent in Coimbatore (Palaniswami, 1978), 30 per cent in Lucknow (Om prakash and Raoof, 1988), 12.5 per cent in Bangladesh (Quroshi and Meah, 1991) and 26.7 per cent in Himachal Pradesh (Sharma
et al., 1993). Stem end rot is a serious post harvest disease of mango spoiling 4-6% fruits every year in Indian market (Pathak et al, 1996). Patil (1992) reported 3.3% loss in mango fruits due to stem end rot. Stem end rot not only spoils 4 to 6 per cent fruits but also adversely affects the export (Singh, 1996). According to Jadeja (2000) stem end rot and Aspergillus niger rot are common post- harvest diseases of mango fruits cv Kesar.

Hasabnis (1984) found pale yellow to dark brown stem end rot symptoms, which appeared within 48 hours, spoiled the fruits within 2-3 days. Philip (2002) observed that initially symptoms are in the form of development of black irregular areas around the pedicel. Soon the affected area enlarges to form circular or irregular patches are also seen scattered all over the surface and within 5-7 days whole fruit become completely black and gets rotten. Under high humid conditions, the rot becomes rapid and grayish black growth of the fungus covers fruit. Due to stem end rot the pulp of fruit become brown and soft. Singh (1996) revealed that stem end rot can be easily recognized by its characteristics symptoms. In the initial stage the epicarp become darken around pedicel base. After few hours the infected area enlarges to form a circular black patch which extends rapidly and turns the whole fruit completely black within 2-3 days, under humid condition and the pulp of the infected fruit become brown and soft. Sharma (2000) reported similar results that it is easy to recognize the stem end rot because it has characteristic symptoms. During initial stage the epicarp darkened around the base of the pedicel, latter it enlarged to form a circular black patch which extends rapidly under humid conditions and whole fruit turns black within 2-3 days. Sharma (2000) also recommended that the pulp in diseased fruit gets soften and become brownish. Recently Gud and Raut (2004) and Gadgile et al. (2009) reported that stem end rot of mango was observed only on ripened fruits. At petiolar region of the fruit, symptoms were developed after 3-4 days, and subsequently the entire fruit got rotten. The pulp of infected fruits became soft, discolored and it gave fermentative
odour. Fungal infection, initiated around the petiole turned black which expanded with age and the whole fruit got affected on ninth day.

*Aspergillus niger* rot (*Aspergillus* rot or Black mould rot)

*Aspergillus niger* van Tiegh was found responsible for *Aspergillus niger* rot in various parts of the country (Srivastava et al., 1965). Mostly the *Aspergillus* rot is caused by *Aspergillus niger* but other species such as *A. fumigatus* and *A. nidulans* are also responsible for *Aspergillus* rot. *Aspergillus niger* rot of mango fruits is disease of wide occurrence. The black mould rot of mango has been reported from Phillipines, Venezuela and India (Das Gupta and Bhatt, 1946; Srivastava et al., 1965 and Srivastava, 1968). Black mould rot is an important market disease of mango fruits (Singh, 2000). Samaia et al. (2006) reported a similar result that is during storage and marketing processes, this disease is more serious. *Aspergillus* rot very common all states of the country and it is responsible for heavy loss of mango fruit. *Aspergillus niger* rot caused 20-22% loss in Totapuri and Langra varieties in Allahabad in June 1964 and 35% loss in Safeda Lucknow variety in Lucknow in July, 1964 (Arya, 1993).

Sangchote (1987) and Arya (1993) observed that *Aspergillus* rot mostly started at stem end while sometime at wounded parts. Arya (1993) also revealed that symptoms first appear in the form of light brown circular patch. The shade of color of infected area depends upon the variety the stage of the disease and the variety of the fruit. The Lesions grows in a regular manner and develop in to large circular spot whereas Philip (2002) reported that black mould rot symptoms appear as grayish or pale brown spots anywhere on the fruit surface, which later on become soft and sunken with a whitish growth.

*Rhizopus* rot (Soft rot)

*Rhizopus* rot of mango fruit is an important transit and market disease. *Rhizopus arrhizus* alone, responsible for 6.3 per cent of fruits in Delhi markets (Thakur and Chenulu, 1966). Thakur (1972) found two more
species responsible for soft rot of mango fruits that is *Rhizopus stolonifer* and *R. oryzae*.

Arya (1993), Sharma (2000) and Sumaia et al. (2006) observed that soft rot appears in the form of light brown colored circular patches on the fruit surface. The intensity of color varies according to the variety and duration of time. The lesions grow in regular manner and form circular patches. The wall of fruit becomes soft and in severe cases, watery ooze comes out.

*Alternaria* rot

*Alternaria alternata* and *A. tenuissima* are responsible for *Alternaria* rot (Philip, 2002). In *Alternaria* rot, initially small water soaked circular brownish spots are develop which enlarge to form irregular patches. Reddish patches develop on the flesh below the spots in the fruit (Aulakh et al., 1998).

Detection of diseases of fruits by non-destructive X-ray imaging technique

It is well known that X-rays can penetrate most materials. In addition to applications in industry and for medical examinations, X-rays have long been used in airport security systems by customs agents to check passengers’ luggage. Yuasa (1926) indicated the importance of the application of X-rays in plant quarantine. Reyes et al. (2000) enabled the use of X-ray images to diagnose the mango seed weevil in intact mango fruit by evaluating distinct features of weevil-infested mango seeds. Thomas et al. (1993), Janave (2007) and Janave and Sharma (2008) reported that spongy tissue of mango fruits weaveled that as detected by the non-destructive X-ray imaging technique. Deshpande (2007) also revealed same results. Yang et al., (2006) found that early Infestations caused by Oriental Fruit Fly (*Bactrocera dorsalis*) (Diptera: Tephritidae) in apple, pear, peach, cherry tomato and orange was detected by x-ray scanning.
Spongy tissue of mango fruits

Alphonso, the most delicious variety of Indian mangoes famous for its excellent aroma and taste, accounts for about 60% of the mango export trade. However, this fruit is susceptible to an internal physiological disease known as ‘spongy tissue’. The diseased fruits do not show any external symptoms and the defect is detected only after cutting, posing a challenge for quality control. In diseased fruits, the pulp adjacent to the seed remains unripe and whitish in colour. The spongy pulp changes colour from faint yellow to brownish black, with or without air pockets accompanied with unacceptable off flavour. The occurrence of spongy defect is prevalent in the coastal Konkan region of Maharashtra State in India, the natural habitat of the Alphonso variety. Non-destructive X-ray imaging detects the spongy tissue-affected fruits (Thomas et al., 1993).

The development of spongy tissue disorder has also been reported from other parts of the country, as well as in other mango-growing regions worldwide. Other varieties in India, namely, Goamankurd, Jamadar, Vanraj, Olour, Vellaikolamben, Swarnrekha and Fernandin grown in southern parts of India also show the presence of this disorder (Katrodia, 1988). This disorder is also prevalent in the Balsad variety of Alphonso mangoes grown in Gujarat state, India (Janave, 2006). The spongy tissue has been also reported in Tommy Atkins mangoes grown in Brazil (Lima et al., 1999 and 2001), Ching Hwang mangoes grown in Taiwan (Lee et al. 2000), cv. Carbao grown in Philippines (Dasuki, 1987), mangoes grown in Malaysia (Lim and Khoo, 1985) and in Florida, USA (Kader, 2006, Paull and Chen, 2002 and Raymond et al. 1998a), and varieties Zill, Sensation and Tommy Atkins grown in South Africa (Oosthuysie, 1997). The disorders of mango, internal breakdown (spongy tissue), jelly seed and soft nose are thought to be one and the same (van Lelyveld and Smith, 1979; Lim and Khoo, 1985). Soft nose disorder does not develop after harvest unless it was present at harvest (Young and Miner, 1961).
In general, about 30% or in extreme cases about 60% of the produce (Vasanthaiah et al., 2006) is affected by this disorder. Also, this disorder has tremendous adverse impact on the export potential of the famous Alphonso variety grown in India. Several investigations have been carried out in the past to understand the causes of spongy defect, but without success. Spongy tissue formation has been associated with factors as diverse as nutritional, ecological, physiological and biochemical (Gupta et al., 1985; Katrodia, 1988; Lima et al., 2001 and Raymond et al. 1998b). Some of the probable causative factors thought to be responsible include deficiency of nutrients such as calcium (Gunjate et al., 1979 and Raymond et al. 1998a), bacterial infection (Chhatpar et al., 1968), late harvesting or the fruits ripened on the tree (Katrodia, 1988), high temperature (Katrodia and Rane, 1989; Ravindra and Shivashankar, 2004), excessive tree vigour (Katrodia and Seth, 1989) and lower fruit transpiration (Shivashankar and Mathai, 1999).

Attempts have been made in the past to control this disorder, but no fullproof solution has yet been achieved. Pre-harvest Ca sprays or post-harvest Ca solution dip treatments have been employed to control spongy tissue (Raymond et al., 1998a and Oosthuyse, 1997). However, the literature shows contradictory findings; in some cases increased incidence of spongy tissue was observed after calcium sprays (Oosthuyse, 1997). The findings by Raymond et al. (1998a) indicated that internal breakdown could not be specifically linked to Ca deficiency at any stage of fruit ontogeny. Storage of the fruits at ambient temperature along with good aeration to prevent excessive heat and to avoid O2 has been employed to reduce the extent of this defect (Dasuki, 1987). Harvesting fruits at 80% maturity has been adopted in certain mango-growing regions in India to avoid spongy tissue. Pre-harvest treatment with paclobutrazol resulted in a considerable reduction of spongy tissue incidence (Ravindra and Shivashankar, 2004). Despite all these treatments, no effective method is available to eliminate
spongy tissue completely, probably due to the fact that the actual causative agent of spongy tissue formation is not yet known.

A recent finding indicated that spongy tissue development was closely associated with the shift of the seed into germination mode (Ravindra and Shivashankar, 2004). The differentially expressed genes related to various enzymes specific to spongy tissue have been cloned (Vasanthaiah et al., 2006). These studies also are largely focused on the biochemical and enzymological changes in the pulp and seed. Despite all the biochemical and enzymological studies, the actual agent responsible for this disorder has not been identified. No report is yet available to correlate spongy tissue development with microbial infection, except by Chhatpar et al., (1968). Bacterial colonies were isolated from spongy tissue (Janave, 2006). The observation was repeated several times observing all the aseptic techniques. This observation indicated an association of a bacterium with the diseased pulp. As a result of this bacterial infection, several biochemical changes such as increased respiration rate, loss of carotenoids associated with lipoxygenase-mediated β-carotene co-oxidation, increase in peroxidase (POX) activity, decrease in soluble protein and increase in total phenolic content were observed (Janave, 2007).

**Impact of physical factors on development of post-harvest fungal diseases of mango fruits**

Temperature and relative humidity percent (R.H. %) are known to exert a powerful impact on fruit rots. These factors retard process of ripening that increase the fruit defense and these factors also affect the various physiological processes of the pathogen during infection and pathogenesis (Patel and Pathak, 1995). Physical factors like temperature and Relative humidity (R.H.) play very important role in the development and spread of post–harvest fungal diseases (Bagwan and Yeole, 2003; Sumaia et al., 2006 and Gadgile et al., 2009). The influence of the temperature and humidity on the decay of mango, banana, guava, papaya, pomegranate, citrus and certain other tropical and subtropical fruits have
been studied by several workers (Srivastava and Tandon, 1968; Thakur, 1972; Kanwar et al., 1973; Gupta and Nema, 1979; Patil and Pathak, 1993; Patel and Pathak, 1995; Jadeja, 2000; Bagwan and Meshram, 2003, Bagwan and Yeole, 2003; Sumaia et al., 2006; Cherian and Varghese, 2007 and Gadgile et al., 2009b). The impact of the environment factors, particularly temperature and humidity play a very important part in determining the nature and activity of the microflora. Thus these factors not have only direct influence on the growth of the fungi, but they can also appreciably affect the fungal advancement indirectly by increasing or decreasing the resistance of the host (Sumaia et al., 2006).

**Temperature**

Establishment and progress of post-harvest fruit diseases is depend upon the availability of a suitable temperature. The optimum temperature which favours the growth and sporulation of a pathogen in *in vitro* culture is generally also suitable for the development and spread of the corresponding fruit rot. (Ghosh, 1998).

Pathak and Srivastava (1969), Prasad and Sinha (1981), Meah et al. (1991) and Patil and Pathak (1993) observed that the fruits incubated at $10^\circ$C did not develop any symptoms of *Botryodiplodia* rot of mango fruit. Highest disease development was recorded in fruits incubated at $25^\circ$C. Spore germination was highest at $25^\circ$C while at $10^\circ$C germination did not occur after 6 and 24 hours. While Patel and Pathak (1995) reported that $30^\circ$ C temperature responsible for severity in *Botryodiplodia* rot of guava fruits. Jadeja (2000) also revealed that $30^\circ$ C temperature was found to be optimum for stem end rot development. Stem end rot hampered at low temperature and it did not develop up to $15^\circ$C. Sumia et al. (2006) found that *Botryodiplodia* rot of mango fruits was severe at $25^\circ$C – $30^\circ$C and at high humidity.

Sharma (2000) observed a temperature of $25^\circ$C has been considered favorable for anthracnose development of post harvest mango fruits. Temperature between 25 to $30^\circ$C was favorable for *C. gloeosporioides* rot.
Prabakar et al. (2003) investigated that the optimum temperature for the anthracnose development was 25\(^0\)C and it was least at 13\(^0\)C. Maximum temperature (>32\(^0\)C) was found unfavorable for anthracnose disease (Patel and Rathod, 2005). Chrys (2006) reported that 25-30 \(^0\)C temperatures were favorable for spore germination of \textit{C. gloeosporioides}. Sumia at al. (2006) found that the temperature between 20\(^0\)C-30\(^0\) C was favorable for \textit{C. gloeosporioides} rot.

Prasad and Sinha (1981) and Bagwavan and Yeole (2003) found that \textit{A. niger} rot in mango fruits is sever at 30\(^0\) C and 10\(^0\) C there is no symptoms of the same. The spore germination of \textit{A. niger} was increased with time and temperature. The spore germination did not occur at 10\(^0\)C upto 24 hours of incubation. Maximum spore germination was at 30\(^0\)C and minimum at 40\(^0\)C. Jadeja (2000) also revealed that 30\(^0\) C temperature was found to be optimum for \textit{A. niger} rot development. \textit{A. niger} rot hampered at low temperature and it did not develop up to 15\(^0\)C. Sumia et al. (2006) found similar results that optimum temperature for development of \textit{A. niger} in mango fruits is 30\(^0\) C. Wardlaw and Leonord (1936), has recommended the storage of mangoes at 10\(^0\)C temperature to avoid fungal infection during storage and room temperature fruits get spoiled within 5-6 days.

Bagwavan and Yeole (2003) and Gadgile et al. (2009 b) found that \textit{R. arrhizus} rot in mango fruits is sever at 35\(^0\) C and at 10\(^0\) C there is no symptoms of the same. The spore germination of \textit{R. arrhizus} was increased with temperature. The spore germination did not occur at 10\(^0\)C up to 24 hours of incubation. Maximum spore germination was at 30\(^0\)C and Minimum at 40\(^0\)C. Thakur (1972) reported that 25\(^0\)C is favorable for development of \textit{Rhizopus} rot. Wardlaw and Leonord (1936), has recommended the storage of mangoes at 10\(^0\)C temperature to avoid fungal infection during storage and room temperature fruits get spoiled within 5 - 6 days.
Relative humidity (R. H.)

Relative humidity has also profound effect on post-harvest rot of fruits in storage. The humidity has direct effect on fungal forms and host. R.H. has an important role to play in the initiation of infection and it is usually essential for the successful initiation of post-harvest fruit decay (Ghosh, 1998). During moist environmental conditions, the affected fruits get covered with huge sporulation of fungi. As the R.H. decreases, the severity of the infection decline sharply (Sumia et al., 2006).

Prasad and Sinha (1981) and Patil and Pathak (1993) observed that at 100% R.H. *B. theobromae* rot severity was maximum. Severity was less at 30% R.H. Severity was increased from 30 to 100% R.H. The spore germination of *B. theobromae* was increased with temperature and R.H. level. Maximum spore germination was at 100% R.H. and minimum at 30% R.H. Patel and Pathak (1995) reported that at 100% R.H. humidity is responsible for severity in *Botryodiplodia* rot of Guava fruits. Sumia et al. (2006) found that *Botryodiplodia* rot of mango fruits was severe at high humidity.

Chrys (2006) reported that 95 % R.H. was favorable for spore germination of *C. gloeosporioides*. Sumia et al. (2006) found that the 95-97 percent was favorable for *C. gloeosporioides* rot. Sharma (2000) found R.H. more than 95 percent has been considered favorable for anthracnose development of post harvest mango fruits. 90 percent R.H. was favorable for *C. gloeosporioides* rot.

Bagwavan and Yeole (2003) found that severity of *A. niger* rot of mango fruits was highest at 100% R.H and lowest at 30 % R.H. Bagwavan and Yeole (2003) also reported that *R. arrhizus* rot in mango fruits is minimum at 30 % R.H. while maximum at 100% R.H. Patil and Pathak (1993) observed that Severity of *Rhizopus* rot was maximum at 100% R.H. Severity was absent at 30% R.H. showed very less rotting of mango fruits. Severity was increased from 30 to 100% R.H. The spore germination of *Rhizopus arrhizus* was highest at 100% R.H. The germination was lowest at
30% R.H. Thakur (1972) reported that 100% R.H. is favorable for development of *Rhizopus* rot.

**Genetic diversity of plant pathogenic fungi by ISSR primers**

Molecular genetics techniques for the DNA fingerprinting of living organisms are proving to be important tools for increasing our understanding of the genetic variability and epidemiology of fungal plant pathogens. DNA fingerprinting is a technique which has been widely adopted in order to differentiate among organisms at the species and subspecies levels (Maclean et al., 1993). DNA fingerprinting techniques have been developed for measuring genetic variability (Emel, 2010). Molecular markers detect more diversity than morphological and biochemical markers as they are polymorphic and are not affected by the environmental conditions and are simply inherited. Molecular markers have been widely adopted to determine the genetic characteristics of fungi, plants and animals. The invention of PCR led to the development of faster and inexpensive molecular markers. Several successful uses of PCR-based techniques have been reported for the identification of fungal pathogen at taxonomic level lower than the species (Gil-Lamaignere, 2003).

Different types of molecular markers have been used to analyse diversity of plant pathogenic fungi at the genome level such as random amplified polymorphic DNA (RAPD) (Duncan et al., 1993; Grazal-Matin, 1993; Loudon, 1993; Quellet and Seifert, 1993; Assigbetse et al., 1994; Bentley et al., 1994; Manulis et al., 1994; Bentley et al., 1995; Lanfranco et al., 1995; Tommerup et al., 1995; Miller, 1996; Wright et al., 1996; Sicard et al., 1997; Brown, 1998; Migheli et al., 1998; Alves-Santos, 1999; Walkers et al., 2001; Abd-Elsalam, 2003; Pasquali et al., 2003; Jana, 2003; Khalil et al., 2003; Weeds et al., 2003; Yi et al., 2003; Beladid et al., 2004; Bastista et al., 2008; Maina et al., 2009; Raju et al., 2009; Pathania, 2010; Mathews, 2010; Saharan, 2010), amplified fragment length polymorphism (AFLP) (Mieadaner and Schilling, 1996; Meng and Chen, 2001; Beladid et al., 2004; Schmidt et al., 2004; Chavan, 2008; Qu et al.,
2008), restriction fragment length polymorphism (RFLP) (Aradhya et al., 2001), simple sequence repeats (SSRs) (Vogelgsang et al., 2009) and inter simple sequence repeats (ISSR) (Pujol Vieira dos Santos et al., 2002; Mishra et al., 2003; Mishra et al., 2004; Tymon and Pell, 2005; Chadha and Gopalkrishna, 2007; Stenglein and Balatti., 2006; Bayraktar and Dolar, 2008; Moreno et al., 2008; Dinolfo, 2010; Mohammadi et al., 2011). ISSR consists of the amplification of DNA sequences between SSR by means of anchored or non-anchored SSR homologous primers (Zietkiewicz et al. 1994). SSRs are tandem repeat motifs composed of one to six nucleotides, which are ubiquitous, abundant and highly polymorphic in most eukaryotic genomes (Tautz and Renz, 1984).

Inter Simple Sequence Repeat (ISSR) is PCR-based technique that is similar to RAPD technique except that the ISSR primer sequences are longer and are designed from microsatellite regions. Therefore, the annealing temperatures used are higher than those used for RAPD markers which could lead to higher consistency of the PCR products. The advantage of the ISSR technique lies in the effective multilocus markers used for diversity analysis, fingerprinting and genome mapping. They are easy to employ and are highly reproducible compared with other techniques such as RAPD, and no prior sequence knowledge is required (Goldwin et al., 1997).

Bornet and Branchard (2001) also reported that ISSR does not require a previous knowledge of the sequence and generates specific and reproducible patterns due to the high stringent conditions of annealing. ISSR markers have been study the population genetics of various eukaryotes and have provided important information relevant to the resolution of different genetic and phylogenetic properties in the range of fungi (Mishra et al., 2003). ISSR have been used in the studies of population genetics and in clonal diversity (Li and Ge, 2001 and Esselman et al., 1999). ISSR markers are more informative than RAPD marker, not only in fungal study but also in plant system (Souframanien and Gopalkrishna, 2004, Nagoaka and Ogihara, 1997 and Raina et al., 2001).
According to Nagaoka and Ogihara (1997) ISSR is a simple technique which is carried out using a single primer based on a simple repeat and only small amounts of DNA template are required and the results are clearly scorable and reproducible. ISSR analysis is a simpler procedure than AFLP analysis because there is no need for an adapter ligation step. In addition, ISSRs appear to be more stable than RAPDs because they have longer primer sequences and use a higher annealing temperature during PCR (McCall et al., 2004). The primers for the repeat regions can be designed outside of the region or within the repeats. Microsatellites and minisatellite primers are reported to be more effective since the sequences are usually dispersed throughout the genome. Just as with repeat sequence probes, variability due to high frequency of change in the sequences may reduce the effectiveness of the method in clustering moderately related isolates (Mbofung, 2006).

The ISSR have been successfully applied to study the genetic diversity of pathogenic fungi e.g. *Sphaeropsis sapinea* (Burgess et al., 2001), *Phialophora gregata* (Meng and Chen, 2001), *Serpula lacrymans* (Kauserud, 2003), *Trichaptum abietinum* (Kauserud and Schumacher, 2003), *Ustilago* spp.(Menzies et al., 2003), *Cryphonectria cubensis* (Van Der Merwe, 2003), *Fusarium graminearum* (Mishra et al., 2004), *Beauveria bassiana* (Elena Estrada, 2007), *F. oxysporum* f. sp. *cicer* (Bayraktar and Dolar, 2008), *Fusarium poae* (Dinolfo, 2010), *Colletotrichum capsici* (Ratanacherdchai et al., 2010), *Fusarium oxysporum* f. sp. *lentis* (Mohammadi et al., 2011), *Corynespora cassicola* (Qi et al., 2011).

Bart-Delabesse et al. (2001), Rath (2002), Myoken et al. (2003), Fungaro et al. (2004) and Abed (2008) studied the genetic diversity among the *Aspergillus terreus*, *A. fumigatus*, *A. ustus*, *A. flavus*, *A. carbonariusi* and *A. niger* isolates respectively by RAPD. Batista et al. (2008) reported the high genetic diversity in *A. flavus* using RAPD and ISSR molecular markers. Hua (2011) also reported the genetic variability in *Aspergillus oryzae* by using RAPD and ISSR.
Hydrolytic enzyme activity

Cellulase and pectinase enzymes action of post-harvest fungi

In storage and market, fruits get infected by several fungi and during their infection bring the changes in the host fruit tissue by modifying their chemical content (Mehrotra et al, 1998). Post-harvest fungi of fruits are specific in their nutritional requirements. Therefore, they attack their respective susceptible host and causes changes in the stored product by absorbing or by hydrolysing complex forms into simpler assimilable substances through the hydrolytic enzyme activity. Pectic and cellulolytic enzymes are the two main biological weapons used by the fungi to break down the pectic and cellulolytic substances of the host cell wall. Several workers have recorded the elaboration of such enzymes e.g. pectinases, hydrolases, oxidases and other enzymes by pathogens and storage fungi causing post-harvest diseases (Wood, 1960).

Impact of nutritional sources on hydrolytic enzyme action

Chapman et al. (1975) reported that starch induces amylase activity of fungi. Among various inorganic nitrogen sources potassium nitrate and sodium nitrate were stimulatory for amylase production of *Aspergillus flavus*, *A. fumigatus* and *Penicillium italicum* (Singh and Agrawal, 1981). Khairnar (1987) reported inhibitory nature in potassium nitrate and sodium nitrate on amylase activity in *Alternaria alternata*. Rathod (2007) reported that disaccharides and polysaccharides inhibited lipase enzyme activity and nitrogen sources as like calcium nitrate, casein, gelatin, peptone increases lipase enzyme activity. Kesare (2009) investigated that nitrogen sources like sodium nitrate, sodium nitrate, ammonium phosphate, ammonium sulphate, urea, gelatin and peptone inhibit lipase enzyme activity whereas, casein stimulates lipase enzyme activity of *Aspergillus glaucus*, *Fusarium roseum* and *Spicaria violecia* while sodium nitrate stimulates lipase enzyme activity of *Curvularia lunata*. Among the phosphorus sources, sodium dihydrogen phosphate inhibited protease activity in *Alternaria alternata*, *Aspergillus niger*, *A. ustus*, *Fusarium roseum* and *Trichoderma viride*. Whereas, it
stimulated protease production in *A. glaucus*, *Curvularia lunata* and *Spicaria violecia*.

Kesare (2009) also found that zinc sulphate inhibits protease activity of *Aspergillus flavus* and *Fusarium roseum*. Sodium thiosulphate completely inhibited the protease activity, whereas lipase production was totally inhibited by ferrous sulphate in *Aspergillus flavus, A. niger, A. glaucus* and *A. ustus*. Ammonium sulphate inhibited *A. flavus, A. galucus, A. ustus* and *Spicaria violecia*, whereas zinc sulphate inhibited lipase activity in *Aspergillus niger*. Kakde and Chavan (2011) found that fructose and sucrose stimulates lipase activity while lactose, carboxyl methyl cellulose and starch inhibited lipase activity.

**Impact of antibiotics, vitamins and fungicides on hydrolytic enzyme action of fungi**

Jayaraman and Prasad (1971) found riboflavin was found to be stimulatory for amylase production of *Aspergillus terreus*. Bhikane (1988) reported that thiamine and nicotinic acid were proved to be stimulatory for protease production in *A. flavus*, while pyridoxine was found to be inhibitory in case of *Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia soloni* for protease production.

Khairnar (1987) found streptomycin and streptopenicillin stimulated amylase production of fungi while hostacycline inhibited the same whereas Rathod (2007) revealed that antibiotics did not affect the amylase, lipase and protease activity of seed borne fungi.

Jadhav (2006) found that a fungicide such as bavistin, benomyl, captan, difoltan, diathane M- 45 and tilt inhibits amylase activity and a vitamin induces amylase activity of fungi on medicinal plants. Among fungicides benomyl and bavistin proved highly inhibitory for amylase action of *Fusarium oxysporum*. On the contrary, hexathir, difoltan, cumin, dithane Z-78 and mancozeb with more and less degree proved inhibitory for amylase production in *Fusarium oxysporum* and *Helminthosporium langstrothra* (Bhosale, 1989).
Impact of physical factors on hydrolytic enzyme action of fungi

Rathod (2010) revealed that alternate light and dark stimulated cellulase and pectinase action in all tested fungi. Maximum cellulase and pectinase activity of all post-harvest fungi was found in between 15-20th days of incubation period. Temperature range between 20-35°C is more suitable for cellulase and pectinase production. At pH 5.0 to 7.0 pectinase and cellulase action of all tested fungi was optimum. Bhosale (1989), Sonwane (2002), Jadhav (2006), Rathod (2007), Kesare (2008) and Kulkarni (2009) find more or less similar findings about the effect of temperature, pH and light on other hydrolytic enzyme of fungi.

Biochemical changes in mango fruits due to post-harvest fungi

Nutritional value of the fruits mainly depends on their quality and quantity of sugars, vitamins and other essential substances. Fruits are considered as the best sources of sugars, amino acids, organic acids, vitamins and other nutrients. During pathogenesis various fungi and bacteria not only blemish, disfigure or cause rot to a number of fruit but the post-infectional biochemical changes reduce their food and market values considerably (Mehrotra et al, 1998 and Arya, 1993).

Changes in sugar content

Most of the fruits are high in sugars. Mango contains 16 to 17 percent sugar in edible portion. Value of fruit greatly depends on the quality and concentration of sugars. Generally, with the maturity of fruits concentration of sugars usually increases. The pathogens which are responsible for spoilage of fruits in storage and transit grow mainly at the expense of various carbohydrates present in fruits. During pathogenesis, complex carbohydrates are converted in to simple carbohydrates due to the production of extracellular hydrolytic enzymes by pathogens consequently there is a simultaneous increase in the concentration of simple sugar such as glucose and fructose, etc. After that the concentrations of these sugars also decrease with an incubation or storage period (Arya, 1993).
Tandon (1970), Pandey et al., (1974), Fush et al., (1980), Reddy and Laxminarayana, (1984) reported the changes in sugars in mango infected by *Aspergillus niger*. They found that there is decrease in sugar of mango fruit due to infection of *A. niger*. Palejwala et al. (1987) reported the *A. niger* is responsible for decrease in total carbohydrate, sucrose, glucose and fructose in mango fruits.

Chaudhary et al. (1980) reported that *Pestalotia anonicola*, *Stachybotrys* sp. and *Trichderma viride* were decrease the total sugar and increase the reducing sugar. Similarly *Cladosporium oxysporum* and *Drechslera rostrata* loquat and capegoose-berry, respectively utilized their total sugar contents within ten days (Singh, 1980). Singh and Sinha (1982) found that *Aspergillus flavus* and *A. parasiticus* cause depletion in total, reducing and non reducing sugars of *Citrus sinensis* fruits similar results were observed by Singh and Sinha (1983) in guava fruits. They found that decrease in total, reducing and non reducing sugars of guava fruit was observed due to *Aspergillus flavus* and *A. parasiticus*. Bilgrami et al. (1983) revealed that there was sharp decline in the level of total, reducing and non reducing sugars of dry fruit during *Aspergillus flavus* infestation. Madhukar and Reddy (1991) revealed the *Pestalotiopsis versicolar* and *Rhizoctonia solani* decrease the reducing sugar content in guava fruit. Verma et al. (1991) revealed the total, reducing and non reducing sugars were greatly reduced by *Aspergillus niger*, *A. fumigatus* and *A. luchuensis* in bael fruits. Recently Sawant and Gawai (2011a) found that *Rhizopus stolonifer*, *Aspergillus flavus*, *Penicillium digitatum*, *Curvularia lunata* and *Fusarium moniliforme* were responsible for decrease in total sugar and increase in reducing sugar content of papaya fruit. Sawant and Gawai (2011b) also reported that *Aspergillus niger*, *Fusarium roseum*, *Rhizopus stolonifer* and *Gleosporium musarum* were decreases the total sugar and increases the reducing sugar content of banana fruits.
Changes in ascorbic acid

Nutritive value of fruits is mainly due to their high vitamins contents especially vitamin C. Anola, guava, mango, papaya and Indian plum are good sources of ascorbic acid. The quantity of vitamin C decreases during storage of healthy fruits but losses of vitamin C were far more prominent when the fruits were infected by fungi (Arya, 1993). Ghosh et al. (1966) reported the vitamin C was totally absent after 8 days in mango fruit tissues infected with Colletotrichum gloeosporioides. Srivastava and Tandon (1966) observed the vianin C content was depleted due Botryodiplodia in Langra and Dashehari varieties of mango fruits. Tandon (1970) found that ascorbic acid of mango pulp was decreased due to A.niger. Vitamin C content of mango fruit was depleted by Phomopsis mangiferae and Phoma exigua (Reddy and Laximinarayan, 1984). Similarly (Arya, 1993) reported the mango fruit infected with Botryodiplodia theobromae showed decrease in vitamin C content. Similar results have been reported in guava (Singh and Tandon, 1971; Madhukar and Reddy, 1991; Bashyal et al., 2009), apple (Bisen, 1974 and Chaudhary et al., 1980), anola (Jamluddin et al., 1974; Reddy and Laximinarayan, 1984 and Sharma and Sumbali, 2009), banana (Prasad, 1977), Jujube (Singh and Sumbali, 2000), citrus (Agrawal and Ghosh, 1979), Musambi (Singh and Sinha, 1982).

Changes in ash, calcium and phosphorous content

Ash contains all minerals. Fruits are rich source of source of minerals like calcium, phosphorous, sodium, magnesium and other minerals needed by the body (Arya, 1993). Verma et al. (1991) reported Aspergillus niger, A. fumigatus and A. luchuensis were slightly decrease the ash content in bael fruits. Recently Sawant and Gawai (2011a) found that ash content of papaya fruit was depleted by Rhizopus stolonifer, Aspergillus flavus, Penicillium digitatum, Curvularia lunata and Fusarium moniliforme Sawant and Gawai (2011b) also reported that Aspergillus niger, Fusarium roseum, Rhizopus stolonifer and Gleosporium musarum were responsible loss in ash content of banana fruits. Rathod (2010) reported that ash,
calcium and phosphorous contents of papaya fruit were decreased by *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, Fusarium moniliformae, Fusarium oxysporum, Penicillium digitatum* and *Rhizopus stolonifer*.

**Biological control**

**Antifungal properties of plant parts extract**

Constant use of fungicidal chemicals causes environmental pollution. Consequently, efforts are under way to finding alternatives to chemical fungicides. Research conducted on the use of plant extracts has opened a new avenue for the control of plant diseases (Shivpuri et al., 1997). Numerous studies have revealed the antifungal properties of plant parts extracts against post-harvest fungi.

Studies on inhibitory effects of a diversity of extracts to control fungi such as *Botrytis cinerea, Glomerella cingulata, Penicillium expansum, C. gloeosporioides, Phomopsis magniferae, Rhizopus stolonifer, Pestalotia psidii* and others, have proved the antifungal potential of plant parts extracts (Pandey et al., 1983; Mohamed et al., 1996; Bong and Min, 1997; Wilson et al., 1997 Bommarito et al., 1998 and Baustista et al., 2000; Baustista et al., 2000 a and Baustista et al., 2002). In these studies growth of the pathogens were affected at some stage in their development. Dargan and Saxena (2002) reported that leaf extract of *Withania somnifera* retarded the growth of post harvest fungal pathogen causing fruit rot of tomato (*Aspergillus niger*). Sonwane (2002) observed that leaf extracts of *Polyalthia longifolia* inhibited the growth of *Alternaria alternata, Aspergillus flavus*, where as *Azadirachta indica* leaf extract inhibited the growth of *Aspergillus flavus* and *Curvularia lunata*.

Aqueous extracts of plants viz., *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* showed strong fungicidal activity against fungi viz., *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani* (Alkhail , 2005). Ahmad and
Abdulgaleil (2005) also reported similar effects of *Magnolia grandiflora* L. extracts against *Alternaria alternata*, *Helminthosporium* spp., *Fusarium oxysprum*, *F. culmorium* and *Rhizoctonia solani*. Prasanna Kumar et al. (2005) revealed the ocimum leaf extracts prevent the post-harvest fungal diseases. Shafique et al. (2006) find similar findings that aqueous extract of *Azadirachta indica*, *Mangifera indica*, *Melia azedarach* and *Syzygium cumini* retard the growth of *Alternaria alternata*. Dharurkar (2007) found that the leaf extracts of *Azadirachta indica* proved highly inhibitory against *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium roseum*, and *Penicillium notatum*, leaf extracts of *Ocimum sanctum* and *Datura strominum* showed antifungal activity. Aqueous extract of *Datura metel*, *Azadirachta indica*, *Ocimum sanctum*, *Lantena camara* and *Parthenium* sp. showed antifungal properties against *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium udum* and *Phomopsis vexans* (Lakpale et al., 2008).

Basha et al. (2009) reported that post-harvest fungal pathogens causing crown rot (*Fusarium moniliforme*) and anthracnose (*Colletotrichum musae*) were almost totally inhibited by treatment with *Candida* sp. in combination with neem leaf extract at 15%. Panchal and Patil (2009) found garlic clove, turmeric rhizome, neem leaf, ginger rhizome and tulsi leaf extract reducing the *Alternaria* fruit rot severity of tomato. Meena et al. (2009) were tested eight plant extract viz. *Ocimum sanctum, Vinca rosea, Azadiracta indica, Aloe barbadensis, Withania somnifera, Azadiracta indica* kernel and *Allium sativum* bulb extract for efficacy in reducing the severity of post-harvest fruit rot (*Pestalotiopsis palmarum*) in guava. They found that these plants extract retarded disease severity of fruit rot of guava. Leaf extracts of *Azadirachta indica*, *Vitex nigundo*, *Jatropha curcas*, *Datura strominum*, *Annona squamosa*, *Murraya koenigii* and *Piper betel* were inhibited the growth of post-harvest fungi of papaya fruit (Rathod, 2010).
Antifungal properties of essential oil

In many plants exhibiting biological activities the greater antimicrobial power lies with the essential oil (Tripathi, 2005). Pathak et al. (1971) reported that mustard oil, castor oil and parafilm oil were effective against Rhizopus rot of mango. Further, Roof and Prakash (1983) found the mustard oil, dalda, groundnut, coconut and linseed control the Aspergillus niger rot of mango fruits. Sinniah et al. (1983) and Vir and Sharma (1985) and Niaz and Kazmi (2005) reported the neem oil showed antifungal activity against Aspergillus spp. Similarly Ansari and Shrivastava (1991) found antifungal activity of eucalyptus oil against Aspergillus flavus. Neem oil at 2-10% retarded the growth of Alternaria alternata, Aspergillus niger and Fusarium oxysporum (Locke, 1995). Sitara et al. (2008) found that neem, mustard and black cumin oil showed against fungicidal activity against Aspergillus niger, Fusarium oxysporum, F. moniliforme, F. nivale, F. semitectum, Drechslera hawiinesis and Alternaria alternata. (Nielsen and Rios, 2000) and Dhingra et al. (2004) reported mustard seed oil also showed antifungal activity. Castor oil treatment showed the less fungal percent disease index in mango fruits. (Prasanna Kumar et al., 2005).

Antifungal properties of plant gums

Marques et al., (1992) revealed the cashew tree gum showed fungicidal properties against the Aspergillus flavus, Penicillium implicatium, Colletotrichum musae and Verticillum sp. While Torkuato et. al., (2004) reported the cassia tree gum has weak antimicrobial activity against yeast (Saccharomyces cerevisiae) but cassia tree gum have no activity against fungi like Lasiodiplodia theobromae, Colletrotichum sp. Dharurkar (2007) found the gum of Azadirachta indica, Acacia Arabica, Ficus belanlysis, Acacia chundra, Mangifera indica, Moringa oleofera inhibited the growth of Alternaria alterana, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum and Penicillium notatum.