

2. Literature Review

A main factor limiting the commercial value of horticultural produces is their susceptibility to post harvest pathogens. Orange, Tomato and pineapple are no exception. A number of diseases caused by several pathogens have been reported to occur in the said plant products *i.e.*, fruits and vegetables. To control post harvest diseases of plants effectively, it is necessary to understand the different pathogens present in the fruits. Recent advances in plant pathology have paved the way for the development on innovative techniques to manage post harvest diseases. Biological control and use of botanicals for control of diseases have also gained importance due to the recent global awareness on negative effect of chemical fungicides and considerable research activity on this area. At the onset of present study, it was considered to review the works of the previous workers in the following paragraphs. For the convenience, the observation has been divided into several subgroups, which are as follows:

- Post harvest disease of fruits and vegetables.
- Post harvest disease of orange.
- Post harvest disease of tomato.
- Post harvest disease of pineapple.
- Post harvest disease control by chemical fungicides.
- Post harvest disease control by botanicals.
- Post harvest disease control by microbial antagonist.

Post harvest disease of fruits and vegetables:

Mainly fungi and bacteria are responsible for the decaying of fruits and vegetables. Decay of fruits and vegetable can be traced to infection at the time of flowering and maturity, harvesting, subsequent handling and storage.

Shikanga *et al.* (2009) reported that *Penicillium digitatum* is a commercially important postharvest pathogen of citrus that is responsible for significant annual global losses. Strains of the fungus, which exhibit strong resistance to widely used synthetic fungicides, are of major concern to the industry. The aim of the study was to investigate the antifungal activities of polar extracts and compounds from *Lippia* species, indigenous to South Africa, against a Guazatine[®]-resistant strain of *P. digitatum*.

Sangeetha and Rawal (2008) reported that Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most important diseases of mango. The nutritional studies were taken up to know the best source of carbon and nitrogen required for the growth and sporulation of the fungus. The study indicated that out of six different carbon sources tried, mannitol was found to be the best source of carbon for the growth followed by fructose and sucrose. Heavy sporulation was observed where maltose was used as carbon source followed by moderate sporulation in fructose and lactose. Among the different nitrogen sources tested, ammonium nitrate supported good growth and sporulation. Potassium nitrate and sodium nitrate also showed good growth but with moderate sporulation.

Rahman *et al.* (2008) a total of seven fungi were identified from the surface of fully matured papaya fruits cv. 'Sekaki' collected from two different fields namely University Agriculture Park, UPM and MARDI, Selangor and a fruit exporter [Seng Chew Hup Kee (M) Sdn Bhd, Kajang, Selangor, Malaysia]. They were identified as *Botryodiplodia theobromae*, *Colletotrichum capsici*, *C. gloeosporioides*, *Fusarium* sp., *Phomopsis* sp., *Rhizopus stolonifer* and *Stemphylium* sp. Among the diseases, the highest incidence ranged from 90 to 98% and severity of 25 to 38% were recorded for anthracnose caused by *C. gloeosporioides* for all three sources, followed by stem-end-rot caused by *Botryodiplodia theobromae*. Pathogenicity test showed that both wounded and unwounded fruits inoculated with conidial suspension of *C. gloeosporioides* developed distinct symptoms of anthracnose after three and five days of inoculation, respectively.

Xiao and Boal (2008) reported that apples (*Malus domestica*) may be kept in cold storage for up to 12 months prior to packing, after harvest. Gray mold caused by *Botrytis cinerea* and blue mold caused by *Penicillium expansum* are common postharvest fruit rot diseases affecting apples.

Xiao and Rogers (2004) reported that during March to July 2003, a postharvest fruit rot was observed on 'Golden Delicious', 'Granny Smith', and 'Red Delicious' apples (*Malus domestica*) sampled from commercial packing houses in Washington State. The disease started at the stem bowl area or the calyx end of the fruit. Decayed fruit was apparently not wounded. Decayed areas were brown and firm. Internal decayed flesh appeared yellowish brown. Pycnidia of a fungus formed on the stem, sepals, or the

surface of decayed fruit. Pycnidia were 0.3 to 0.7 mm in diameter, black, and partially immersed in decayed tissues. The fungus was identified as *Sphaeropsis pyriputrescens*.

Deka *et al.* (2006) reported that the post harvest loss of banana is highest (22.00%) among all the fruits in the state of Assam. Claudia *et al.* (2004) reported that the fungus *Colletotrichum musae* can cause both crown rot and anthracnose. In addition, crown rot diseases may also be caused by *Fusarium*, *Acremonium*, *Verticillium*, and *Curvularia*. The two primary postharvest rots of banana fruits are crown rot and anthracnose. The diseases usually appear on ripening fruits either at the point of sale or later after purchase. *Lasiodiplodia theobromae*, *Thielaviopsis paradoxa*, *Colletotrichum musae*, *C. gloeosporioides*, *Fusarium verticillioides* and *F. oxysporum* are few pathogens caused postharvest diseases in Banana.

Ramma *et al.* (1999) identified that crown rot disease was the major post-harvest disease on ripening bananas in Mauritius followed by anthracnose and blossom end rot. Crown rot was found to be due to a complex of fungal pathogens, namely *Fusarium* sp., *Colletotrichum* sp., *Verticillium* sp and *Cladosporium* sp. It was found to develop rapidly during fruit ripening thereby reducing the quality and marketability of fruits.

Liu (1986) reported that Anthracnose (*Colletotrichum* sp.) is one of the main disease causing decay and commercial devaluation of harvested mangoes. He suggested that pathogenic fungi such as anthracnose on mango fruit are latent and a main cause of serious decay.

Al-Rahamah *et al.* (2003) studied the post harvest diseases of apple, orange and lemon fruits collected from markets of Riyadh. They recorded following pathogenic organisms: *Alternaria alternata*, *Aureobasidium pullulans*, *Diplodina* sp. and *Fusarium tricinctum* from apple fruits; *A. alternata*, *Geotrichum candidum* and *Penicillium* sp from orange fruits; *A. alternata*, *A. citri* and *G. candidum* from lemon fruits. The efficacy of some fungicides and chemicals was tested against the post harvest diseases caused by these pathogens. All the tested fungicides and chemicals were less effective against the diseases caused by the two species of *A. alternata* and *G. candidum*.

Snowdon (1992) demonstrated that post harvest rots of watermelon caused by *Fusarium* sp. and *Phytophthora capsici* are of concern because control measures for these fungi in the field are inadequate. It was also reported that good disease control in the field

rarely allow *Colletotrichum orbiculare* and *Didymella bryoniae* to cause anthracnose and black rot respectively on watermelon.

Suslow (1999) reported that in production areas with high RH and temperature, an extensive list of lesions, stem-end or blossom-end rots, and surface lesions may be caused by *Erwinia* or an assortment of fungi (Snowdon, 1992).

Rushing *et al.* (1999) demonstrated that watermelon fruit blotch (*Acidovorax avenae subsp. citrulli*) was a postharvest problem for several years, but research demonstrated the disease is not easily transmitted from fruit to fruit after harvest. Appropriate grading and temperature management can virtually eliminate its presence in the marketing chain.

Seemuller (1998) reported about two of the most important pathogens *Phytophthora fragariae var. fragariae* and *Phytophthora cactorum*, which are the cause of red core and crown rot disease of strawberry, can cause substantial economical damage in strawberry production.

Schwartz and Mohan (1995) observed that in storage conditions, a number of fungi, including *Fusarium* spp., *Botrytis* spp., and *Aspergillus niger*, were present on diseased onion bulbs. Some opportunistic bacteria were associated as endophytic microflora with naturally infected onion bulbs from farms and packing sheds. The microflora includes *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus cereus*, *Erwinia* sp., *Klebsiella* sp., *Enterobacter* sp. and *Escherichia* spp.

Jones and Aldwinckle (1990) reported that blue mold and gray mold, caused by *Penicillium expansum* Link and *Botrytis cinerea* Pers., respectively, are the most damaging postharvest diseases on apples.

Morton (1987) reported that the main post-harvest problem of lychee is spoilage by the yeast-like organism, which is quick to attack warm, moist fruits.

Alvarez and Nishijima (1987) reported that the major postharvest diseases of papaya are anthracnose and stem end rot. Postharvest diseases, especially anthracnose, become a problem when fruit have 25% or more skin yellowing.

Papaya diseases greatly increase in severity and incidence following 4 weeks storage at 10 °C (50 °F). Mechanical injury and chilling injury can enhance development of postharvest disease incidence. *Rhizopus* requires breaks in the cuticle for the disease to

occur. Cuticle disruption occurs as latex vessels break down, when the fruit is 40 to 60% yellow. Fruit fly punctures can also increase *Rhizopus* rot, as can mechanical injuries and lesions caused by fungi such as anthracnose and *Cercospora* black spot (Nishijima *et al.*, 1990).

Mayberry and Hartz (1992) reported that development of fungal rots is key factors in postharvest deterioration of melons. In Melon, postharvest decay mainly occurs from development of the fungal pathogens *Fusarium* spp., *Geotrichum* spp., *Rhizopus* spp., *Cladosporium* spp., *Alternaria* spp. and the bacterial pathogen *Pseudomonas* spp. They also been estimated that the average disease loss after transport to distant markets on the east coast was 30-50% and was even reached 80% in extreme cases.

Heavy postharvest losses may occur due to cultivar susceptibility to disease, rough handling after harvest, inadequate packaging and temperature management and long transport times.

Romano *et al.* (1983) reported that Apple is one of the most important fruits produced. Postharvest losses caused by fungal diseases are the major factors limiting the storage life of apples. Postharvest fungal diseases of apples are mainly caused by *Penicillium expansum*.

Salunkhe and Desai (1984) observed that Gray Mold (*Botrytis cinerea*) rot, Green Mold (*Penicillium digitatum*) rot and *Cladosporium* spp. are the main postharvest diseases of pomegranate fruit. Gray Mold usually starts at the calyx. As it progresses, the skin becomes light-brown, tough and leathery. Heart rot is another disorder that may be caused by *Aspergillus* spp. and *Alternaria* spp. Affected fruit show slightly abnormal skin color and a mass of blackened arils; disease develops while fruit are on the tree.

Post harvest diseases of Orange

Citrus is one of the major commercial fruit that is widely consumed both as fresh fruits and juice. Its global demand attributed to its high vitamin content and high antioxidant potential (Gorinstein *et al.*, 2001). Citrus is mainly cultivated in sub tropical and tropical regions of the world in over 137 countries and six continents (Ismail and Zhang, 2004).

Orange (*Citrus reticulata*) is attacked by several plant pathogens and that affect its fruit quality. In developing countries, where protection and proper handling is

inadequate, loss during transit and storage can represent in excess of 50% of the harvested crops (Eckert and Ogawa, 1985; Wisniewski and Wilson, 1992). Major post harvest losses have been recorded with a range of pathogen.

Blue mold caused by *Penicillium italicum* is one of the most destructive postharvest diseases of citrus fruits (Caccioni, 1998; Palou 2002).

Smilanick *et al.* (2008) reported that green mould (*P. digitatum*) and blue mould (*P. italicum*) account for most of the decay of citrus fruit worldwide. Preharvest infections include *Diplodia natalensis*, *Phomopsis citri*, *Colletotrichum gloeosporioides*, *Phytophthora* species and *Alternaria citri* and post-harvest infections include *Penicillium digitatum*, *P. italicum* and *Geotrichum citriaurantii*.

El-Ghaouth *et al.* (2002) reported that post harvest diseases are mainly caused by fungal pathogens such as *Phytophthora spp*, *Colletotrichum gloeosporidies*, *Botrytis cinerea*, *Diplodia natalensis*, *Phomopsis citri*, *Alternaria citri*.

Brown and Eckert (2000) reported that Sour rot, caused by *Geotrichum citri-aurantii*, is second in importance only to *Penicillium* decays as a wound-mediated disease of citrus. They reported that in most citrus growing areas the diseases occur on all cultivars. It is particularly troublesome on fruit held in long-term cold storage. Sour rot is one of the most serious disease problems for the lemon industry of California due to the long storage of this fruit (Eckert and Brown, 1986). The fungus only infects fruit through injuries and in particular deep injuries that involve the albedo tissue. Sour rot develops more frequently on mature to over-mature fruit with high peel moisture. The initial symptoms were water soaked lesions, light to dark yellow and slightly rose, with the cuticle being more easily removed from the epidermis than lesions caused by green or blue mould. Decayed fruit tissue had a sour odor that attracted fruit flies and those could spread the fungus to other injured fruit during storage. The fungus was present in soil and could reach the fruit surface from wind blown or splash dispersed soil and by fruit-soil contact. Contaminated fruit could spread the pathogen through drenching equipment, soak tanks, pallet bins, washer brushes, belts and conveyors. Uninfected fruits within the container were affected by the infected fruits and spreaded the disease. The disease developed rapidly at warm temperatures, with an optimum of about 27°C.

The major post-harvest diseases in orange could be separated into two categories based on their initial infections. Preharvest infections include *Diplodia natalensis*, *Phomopsis citri*, *Colletotrichum gloeosporioides*, *Phytophthora* species and *Alternaria citri* and post-harvest infections include *Penicillium digitatum*, *P. italicum* and *Geotrichum citriaurantium* (Brown and Miller, 1999).

Droby *et al.* (1989) reported that orange was attacked by several pre/post harvest pathogens that effect fruit quality. Green and blue mold infections caused by *Penicillium* spp, anthracnose caused by *Colletotrichum gloeosporidies* and sour rot caused by *Geotrichum candidum* (Chalutz and Wilson ,1990) are some of the major postharvest problems that causes market losses.

Timmer *et al.* (2000) reported that Brown rot, caused by *Phytophthora palmivora*, *P. citrophthora*, *P. nicotianae*, *P. hibernalis* and/or *P. syringae*, occurs in several citrus-growing regions. In Florida, brown rot was caused by *P. palmivora* and *P. nicotianae*. In addition to causing preharvest fruit losses, brown rot can also result in substantial post-harvest losses. *Phytophthora* species persist in the soil and are spread through rain splashes to fruit hanging on the lower canopy of the trees thereby infecting the fruit. Most infections develop on the tree within 3 to 4 feet of the soil surface although they might be found in higher locations as a result of wind-driven rains. Initial infection shows as light discoloration on any area of the fruit surface. As the decay developed, the lesions became light brown, firm and leathery. Under humid conditions, decayed areas spreaded rapidly and white mycelia might formed on infected areas. Fruit with brown rot had a characteristic rancid odor. Brown rot spreads in packed containers from infected to healthy fruit.

Post harvest diseases of Tomato

Tomatoes are subject to several post harvest diseases and disorders. Proper handling, grading, and temperature management will minimize the occurrence of these potentially devastating problems.

Hadizadeh *et al.* (2009) reported that tomato is susceptible to various post harvest diseases caused by various pathogenic fungi. *Alternaria alternata* is a saprophytic pathogen of tomato causing (*Alternaria* rot) post harvest losses at high frequency.

Pose (2009) reported that *Alternaria alternata* is a toxigenic fungus, predominantly responsible for black mould of ripe tomato fruits, a disease frequently causing substantial losses of tomatoes, especially those used for canning. The objective of this study was to determine the effect of water activity (a_w , 0.904, 0.922, 0.954, 0.982) and temperature (6, 15, 21 and 35 °C) on germination and radial growth rate on a synthetic tomato medium of a cocktail inoculum of five strains of *A. alternata* isolated from tomato fruits affected by blackmould. The knowledge on the ecophysiology of the fungus in the substrate was necessary to elaborate future strategies to prevent its development and evaluate the consumer health risk.

Dal-Bello (2008) reported that the fungal pathogen *Botrytis cinerea* cause severe rots on tomato fruits during storage and reduced shelf life.

Howell *et al.* (2005) reported that Anthracnose of tomato was primarily a disease of ripe and over-ripe fruit. If left unchecked, the disease could cause serious losses in yield and marketability. Caused by several species of *Colletotrichum*, the disease was widespread and common in areas where moisture conditions promote disease development and also affects eggplant, pepper, and potato. *C. coccodes* was the most common pathogen of tomato fruit.

Tomatoes can develop many postharvest diseases including Alternaria rot (*Alternaria alternata*), gray mold or Botrytis (*Botrytis cinerea*), rhizopus or hairy rot (*Rhizopus stolonifer*), and sour rot (*Geotrichum candidum*). Bacterial soft rot caused by *Erwinia* spp. can be a serious problem if good harvest and packinghouse sanitation practices are not implemented. Wounds and stems and stem scars provide potential points of entry for pathogens and decay organisms, so wounded fruit should be discarded immediately (Anonymous, 2003).

Tohamy *et al.* (2004) found that postharvest decay is the major limiting extension of shelf life in tomato fruits (*Lycopersicon esculentum* Mill.).

Alternaria alternata and *Botrytis cinerea* causing black and grey moulds are the two main fungi responsible for storage decay in Egypt (El-Essawy *et al.*, 2003)

Fajola (1979) studied post-harvest fruit rot diseases of tomato in five states of Nigeria. During severe infections, the diseases could cause 25% loss at harvest and 34% loss of the remaining product in transit, storage and market stalls; thus giving an overall

loss of about 50% of the product. Two types of rots, soft and dry were recognised. The soft rot was found to account for about 85% and the dry rot about 15% of the overall loss. *Erwinia carotovora*, *Rhizopus oryzae*, *R. stolonifer*, *Fusarium equiseti*, *F. nivale* and *F. oxysporum* were established as the soft rot pathogens; while *Aspergillus aculeatus*, *A. flavus*, *Cladosporium tenuissimum*, *Corynespora cassicola*, *Curvularia lunata*, *Penicillium expansum* *P. multicolor* and *Rhizoctonia solani* were established as the dry rot pathogens of tomato fruits in Nigeria.

Barnett and Hunter (1998) reported that *Cladosporium* species and *Alternaria alternata* identified by microscopic observations were the prevalent fungi (92%). Those pathogens were recovered following surface disinfection of the calyces, indicating that they had colonized the truss as latent disease already in the greenhouse. During storage (12°C), the mycelia expand gradually from the sepal tips to the calyces and then to the peduncles and, finally, to the rachises, all of which eventually shrivel or dehydrate while uninfected trusses remain fresh and green. The severity of the PHECD syndrome increased during the warm seasons and declined in the winter. *Cladosporium* isolates from overtly infected calyces were identified as *C. sphaerospermum* (Penzig) and *C. tenuissimum* (Cooke) by the CBS Identification Service, Netherlands.

Srivastava and Tandon (1966) reported that a systematic account of nine fungal diseases of tomatoes caused by *Alternaria tenuis*, *Colletotrichum dematium*, *Cladosporium fulvum*, *Fusarium roseum*, *Malustela aeria*, *Myrothecium roridum*, *Oospora lactis f. parasitica*, *Phoma* sp. And *Rhizopus nigricans* during storage has been given. All the diseases except *Oospora* rot were reported as new from India and post-harvest decay of tomatoes due to *Malustela aeria* was described as new report. Jones *et al.* (1993) reported that tomato commercialization was limited by rotting caused by *Alternaria alternata* and/or by *Botrytis cinerea*. *Alternaria alternata* is a saprophytic pathogen of tomato causing post harvest losses at high frequency

Oladiran and Iwu (1993) reported that seven fungi associated with fruit rot of tomato. The fungi were *Fusarium equiseti*, *F. chlamydosporum*, *Alternaria solani*, *Geotrichum candidum*, *Acremonium recifei*, *Aspergillus flavus* and *A. niger*. They were all pathogenic on tomato fruits, most pathogenic being *Geotrichum candidum* followed by *A. niger*. Least rot was caused by *Alternaria solani*. The optimum temperature for

maximum rotting caused by *G. candidum*, *A. niger* and *A. flavus* was 30°C. The relative humidity for maximum rot ranged from 70–90%. Tomato fruits stored well at 0–10°C and rather poorly at 20–30°C. Fruits stored at 35°C showed blemishes. The best RH for storage ranged between 60 and 90%.

Howard *et al.* (1994) reported that symptoms were first noticeable on ripe fruit of tomato, although green fruits were infected. The latent infections on green fruits can become a serious post-harvest problem. They also reported that tiny lesions may also occur on leaves and stems, but are usually overlooked. Those lesions serve as initial source of inoculum for fruit infection. Small, circular, sunken spots appear on ripe fruit and are characterized by numerous submerged, black microsclerotia often in concentric rings. Spots can coalesce and involve large areas of the fruit. Under humid conditions, spots darken due to the production of hairs (setae) on the fruiting bodies of the pathogen and pink, gelatinous masses of conidia may ooze from lesions. Lesions may crack and become invaded by secondary soft-rotting organisms.

Sommer *et al.* (1992) observed that *Alternaria* spp., *Cercospora* spp., *Colletotrichum* spp., *Aspergillus* spp. and *Fusarium* spp. were responsible for diseases. It is somewhat similar to postharvest diseases symptoms of tomato.

Narain and Rout (1981) reported that *C. tenuissimum* was associated twice with dry rot of tomato fruit. They also first time reported that two *Cladosporium* species and *A. alternata* promoted the shriveling of tomato calyces and rachises.

Post harvest diseases of Pineapple

Post harvest disease of pineapple (*Ananas comosus*) is economically important in the production of the fresh and canned fruit. Diseases of pineapple include plant as well as fruit problems. Various diseases have been reported to be causing severe losses in pineapple and they have been considered as one of the constraints for low yield of pineapple in several areas. Major pineapple diseases and their causal organisms are as follows:

- i. Heart and root rot (*Phytophthora cinnamomi*)
- ii. Heart rot (*P. parasitica*)
- iii. Root rot (*Pythium* spp.)
- iv. Black rot (*Ceratocystis paradoxa*)

- v. Butt rot (*Thielaviopsis paradoxa*)
- vi. Fruitlet core rot (*Penicillium funiculosum*, *Fusarium moniliforme* var. *subglutinans*)
- vii. Pink disease of Fruit (*Acetomonas* spp.)
- viii. Pineapple mealybug wilt (probably virus)
- ix. Bacterial diseases (*Erwinia carotovora*, *E. chrysanthemi*)
- x. Nematodes (*Meloidogyne* spp., *Rotylenchulus reniformis*)

Wijesinghe *et al.* (2008) reported that black rot, caused by *Thielaviopsis paradoxa*, is the most common and well known postharvest disease of the pineapple fruit. Infection takes place, usually, through wounds in the base of the fruit resulting from harvesting. The pathogen gains entrance also through natural growth cracks and wounds on the fruit surface generated during picking or packing. After penetration through the cut stem end the pathogen grows rapidly upwards and slowly into the flesh resulting, initially, in a dark yellow cone shaped soft rot. Diseased tissue turns dark in the later stages of the disease because of the dark mycelium and spores.

Wilson *et al.* (2005) reported that Black rot of pineapple, caused by *Chalara paradox* is a postharvest disease responsible by high losses on fruits destined to the fresh market and to the processing industry.

Reyes, *et al.* (2004) reported that Incidence and severity of black rot (*Chalara paradoxa*) of pineapple fruit were evaluated on fruit harvested every 2 weeks for 14 months. Rainfall did not play a major role in black rot incidence and severity. Total microbial counts on the fruit were correlated to rainfall in the month of harvest.

Rohrbach and Schmitt (1994) reported that black rot, water blisters, soft rot or water rot is universal fresh pineapple problem characterised by watery soft rot. Black rot is caused by the fungus *Chalara paradoxa*. Fruit core rot, black spot, fruitlet brown rot, and eye rot describe the brown to black color of the central part of an individual fruitlet. Saprophytes like penicillium sp, growing on the broken end of peduncle and fruit surface are non pathogenic creates a problem in marketing.

Rohrbach and Schmitt (1994) reported black rot was caused by the fungus *Chalara paradoxa*. Infection occurs within 8 to 12 h following harvest and enters through the point of detachment or wounds. The severity of the problem was reported to be



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dependent on the degree of bruising or wounding during harvesting and packing, the level of inoculum on the fruit, and storage temperature during transportation and marketing.

Rohrbach and Schmitt (1994) fruitlet core rot, black spot, fruitlet brown rot and eye rot describe the brown to black color of the central part of an individual fruitlet. Epidemic levels are rare in the major commercial pineapple-producing areas of the world. Low-acid cultivars being grown commercially are most susceptible. This disease was caused by a complex of fungi. Infection frequently can lead to misshapen fruit that are culled before packing and shipping.

Zhou, (1993) and Tang, (1995) reported the effect of exogenous gibberellic acid and chilling temperature on the cause and development of blackheart in pineapple and demonstrated that blackheart was mainly due to the catalysed oxidation of phenolic compounds by PPO and that low temperature or gibberellic acid markedly increased PPO activity and catechol, chlorogenic acid, and caffeic acid to accelerate the blackheart.

Rohrbach and Phillips (1990) reported that the symptoms of the post-harvest diseases of pineapple fruit may develop externally or internally. Diseases of pineapple fruit can be classified as: 1) pre-flower infections by *Penicillium funiculosum* that begin at the floret before the flower opens and cause interfruitlet corking, leathery pocket, and fruitlet core rot, 2) flower infections beginning after the flower opens and caused by bacterial complexes including pink and marbling disease, 3) wound infections caused most frequently by *Ceratocystis paradoxa* that begin in wounds caused by harvesting, and 4) physiological disorders the most common of which is internal browning or black heart caused by chilling injury. Currently, *Ceratocystis* fruit rot is controlled by fungicide applications and internal browning by post-harvest wax dips.

Rohrbach and Phillips (1989) reported that postharvest pineapple diseases which develop externally or internally are classified as preflower infections by *Penicillium funiculosum* that begin at the floret before the flower opens and cause interfruitlet corking, leathery pocket and fruitlet core rot. Flower infections beginning after the flower opens and caused by bacterial complexes including pink and marbling disease. Wound infections caused most frequently by *Ceratocystis paradoxa* that begin in wounds caused by harvesting and physiological disorders. The most common is internal browning or

black heart caused by chilling injury. *Ceratocystis* fruit rot is controlled by fungicide applications and internal browning by postharvest wax dips.

Py *et al.*, (1984) reported that Black rot, caused by *Thielaviopsis paradoxa*, is the most common and well known postharvest disease of the pineapple fruit. Infection takes place, usually, through wounds in the base of the fruit resulted from harvesting. The pathogen gains entrance also through natural growth cracks and wounds on the fruit surface generated during picking or packing. After penetration through the cut stem end the pathogen grows rapidly upwards and slowly into the flesh resulting, initially, in a dark yellow cone shaped soft rot. As the disease develops, the flesh becomes watery and the juice exudes, resulting in an empty shell showing only dark fibers inside. The development of black rot in pineapple fruits is very fast at 25°C and very slow at 8°C. Due to that, severe rot problems may occur when untreated fruits are removed from refrigerated conditions and held at room temperature in retail market.

Mourichon (1983) reported that Leathery pocket is a pineapple disease characterized by the suberization of the surface of the locules of the ovary due to the infection by *Penicillium funiculosum*.

Rohrbach (1983) reported that black rot (*Thielaviopsis* fruit rot), water blister, soft rot or water rot are universal fresh pineapple problem. Diseased tissue turns dark in the later stages of the disease because of the dark mycelium and spores.

Rohrbach *et al.* (1981) reported that the pineapple fruit mite (*Steneotarsonemus ananas* Tyron), has been reported to be associated with fruitlet core rot and it seems to enhance the pathogenesis of the fungi that cause the disease. The incidence of fruitlet core rot is influenced by the rainfall that is important for inoculum build up and by the mean daily temperature, that ranging between 16 to 20° C favors mite population and infection by *P. funiculosum*. The pathogen enters the developing inflorescence through unopened buds. It may also enter through the stylar canals and nectary ducts as well as through insect injuries and natural growth cracks.

Post harvest control by chemical fungicides:

Fungicides are the primary means of controlling post harvest diseases. The current methods for managing stored grain pests depend heavily on chemical pesticides. However, repeated use of certain chemical fungicides in packing houses has led to the

appearance of fungicide resistant populations of storage pathogens. Further, the use of synthetic chemicals to control post harvest biodeterioration has been restricted due to their carcinogenicity, teratogenicity, high and acute residual toxicity, hormonal imbalance, long degradation period, environmental pollution and their adverse effects on food and side effects on humans (Brent and Hollomon, 1998; Dubey *et al.*, 2007; Kumar *et al.*, 2007).

Dubey *et al.* (2008) reported that Synthetic fungicides are used as the primary means for the control of plant diseases. Sharma and Meshram (2006) reported that the use of synthetic chemicals as antimicrobials for the management of plant pathogens. The chemicals increased crop protection but with some deterioration of environmental quality and human health.

Poppe *et al.* (2003) reported that traditionally, the postharvest diseases have been controlled by spray of synthetic fungicides such as thiabendazole, imazalil and sodium ortho-phenyl phenate.

Fan (2000) reported that disease control could be achieved mainly through the use of fungicides. Benomyl and iprodione were available for postharvest treatment to reduce decay and extended the shelf-life of stone fruits.

Sticher (1997) reported that chemically induced resistance against a wide variety of pathogens attack have been described for many plant species against a wide variety of pathogens ranging from oomycetes, fungi, bacteria and viruses.

Okonkwo and Okoye (1996) found that the use of synthetic fumigants is also another way to control post harvest disease. But it also led to increased cost of application, pestresistance, lethal effects on non target organisms and toxicity to user.

Wilson and Wisniewski (1994) reported that post harvest disease often account for a major part of the losses and their control requires use of a large amount of fungicides.

Jeffries and Jeger (1990) reported that losses due to postharvest diseases of fruits and vegetables could reach proportions of 10-30% according to the species and kind of technology used. That had a considerable economic impact world-wide, of particular concern in third world countries. Fungi (*Penicillium digitatum*, *Penicillium italicum* and *Botrytis cinerea*) which attack the fruits through wounds or latent preharvest infections generally cause disease. Synthetic chemical compounds are usually employed in the

prevention of alterations but their use has determined the selection of resistant pathogen strains, and thus a reduction in their efficacy.

Lindsay (1985) reported that Bicarbonates at concentrations under 2% are widely used by the food industry to avoid fermentation, to control pH, and to develop satisfactory textures and flavors. At the same time, they are effective against food bacterial and yeast infections and are important in controlling buccal pathogens.

Homma *et al.* (1981) reported that Sodium, potassium, and ammonium bicarbonate made it possible to control some fungal infections of the cucumber during preharvest. Similar treatments with sodium and potassium bicarbonate showed adequate control of *Rhizoctonia carotae* Rader in carrots during post-harvest and controlled post-harvest rotting of citrus fruits, melon (Aharoni *et al.*, 1997) and pepper (Fallik, *et al.*, 1997).

Postharvest disease control by microbial antagonist

Plant diseases occur on a regular basis in plants and cause rigorous economic loss. Application of chemical fungicides leads to destroy beneficial microbes on the crop milieu and thus alters the crop scenario and also causes toxicity to human and natural biota (Patro *et al.*, 2008). Biological control of plant diseases involves the use of one nonpathogenic organism to control or eradicate a pathogenic organism. Hence, biological control has attracted a great attention in plant pathology (Goto, 1990) and it becomes important to develop cheaper management practices to control the post harvest disease. To extend biological control strategies for controlling any disease, a thorough knowledge of life cycle of the pathogen(s), their mode of survival, the plant-pathogen interaction processes, the physical relationship of the pathogen to its host during pathogenesis, the time of infection, factors leading to infection and disease development are needed. Several authors have reported antagonistic activity of microorganisms in different crops (Droby *et al.*, 1992; Prasad *et al.*, 1999; Meena *et al.*, 2000; Dwivedi and Johri, 2003; Jadeja 2003; Kohli and Diwan, 2003; Vestberg *et al.*, 2004; Brewer and Larkin 2005; Sudha *et al.*, 2005; Singh and Sinha, 2005). There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Several authors have demonstrated the use of botanicals to control the plant diseases.

Kotan *et al.* (2009) reported that antagonistic activity of 24 selected bacterial strains detected by microbiological studies to *Aspergillus flavus* and was tested *in vitro* and *in vivo* conditions. Both cell suspension and culture filtrates of prominent 10 bacterial strains were also tested in order to control *A. flavus* on lemon fruits cvs Meyer and Interdonato under storage conditions. The cell suspension of nine strains and the culture filtrates of three strains led to suppression on disease development on lemon fruits. The highest control was obtained by the cell suspension of *Pantoea agglomerans* RK-153. *Erwinia chrysanthemi* RK-67 and *Bacillus subtilis* RK-6 treatments reduced disease severity on both lemon cultivars. Furthermore, both the cell suspension and culture filtrates of *Burkholderia cepacia* strain RK-277 reduced disease severity on only cvs Interdonato, but not on Meyer.

Marikar *et al.* (2008) reported that *Trichoderma harzianum*-TrH40 isolated from soil samples from rambutan (*Nephelium lappaceum*) orchards had antagonistic effects with combination of CaCl_2 salts on growth, activity, and infection of rambutan fruits by anthracnose (*Colletotrichum gloeosporioides*). Furthermore, disease incidence and severity with combined treatments were correlated significantly and effectively reduced infection *in vivo* in rambutan fruits.

Zhang *et al.* (2007) reported that *Cryptococcus laurentii* was evaluated for its activity in reducing postharvest gray mold decay, blue mold decay and Rhizopus decay of peach caused by *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* respectively, and in reducing natural decay

Rabosto *et al.* (2006) searched for biological agents capable of controlling the phytopathogenic fungus *Botrytis cinerea*, winegrape cuticles of several *Vitis vinifera* varieties and different soils from Uruguayan vineyards were screened for microorganisms that could be antagonistic to the fungus. Among 223 isolates of yeasts and bacteria, eight non-*Saccharomyces* yeast strains and four bacteria showed greater than 50% effectiveness *in vitro* against *Botrytis cinerea* compared with the growth of the control. A species of *Bacillus* (isolate UYBC38) and an isolate of the yeast *Hanseniaspora uvarum* (isolate UYNS13) showed high *in vitro* antagonistic capability against the pathogen; both organisms were very effective (100% and 90%, respectively) in controlling rot development on grape clusters. The possible mechanisms

of antagonism against *B. cinerea* were studied. Yeast strain UYNS13 showed only competition for nutrients. Although the *Bacillus sp.* strain consumed significantly more nitrogen from grape juice than *B. cinerea*, the relevant mechanism of antagonism of this bacterium was the production of anti-fungal substances. These compounds completely inhibited germination of *B. cinerea* conidia *in vitro* by disrupting the conidial cell wall, resulting in leakage of protoplasm. Applications of UYBC38 in the vineyard showed that this strain could survive on leaves and fruits for 45 to 70 days after spraying.

Nourozian *et al.*, (2006) reported that *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces sp.* strain 3 were evaluated as potential biological agents for control of fusarium head blight (FHB) caused by *Fusarium graminearum*.

Zheng *et al.* (2005) investigated *in vitro* and *in vivo* biocontrol activity of *Rhodotorula glutinis* on green mold decay of orange caused by *Penicillium digitatum*. Significant control was achieved with a washed cell suspension and unwashed cell culture mixture of *R. glutinis*. They also evaluated *Cryptococcus laurentii* for its activity in reducing postharvest blue mold decay of oranges caused by *Penicillium italicum in vitro* and *in vivo*. They showed that washed cell suspensions of yeast provided control of blue mold decay better than yeast in culture broth.

Nunes *et al.* (2001) Epiphytic microorganisms isolated from the fruits and leaf surfaces of apples and pears were screened for antagonistic activity against *Penicillium expansum* on pears. From 247 microorganisms tested for antagonistic properties against *P. expansum*, a bacterium strain identified as *Pantoea agglomerans* (CPA-2) was selected. The bacterium was very effective against *Botrytis cinerea*, *P. expansum* and *Rhizopus stolonifer*. In over 3 years of experiments in semicommercial trials, *P. agglomerans* provided excellent control against *B. cinerea* and *P. expansum* under cold storage, either in air or in low oxygen atmospheres. Equal control was obtained with *P. agglomerans* at 8×10^7 CFU ml⁻¹, as with the fungicide imazalil at commercial doses, against both the pathogens. *P. agglomerans* grew well inside wounds on pears at both room and cold temperatures and under modified atmospheres. In contrast, it grew poorly on the surface of intact fruit.

D'souza *et al.* (2001) reported that *Trichoderma harzianum* has antagonistic effect against four fungal pathogens (viz. *Phytophthora parasitica*, *Colletotrichum*

capsici, *Sclerotium rolfsii* and *Rhizoctonia solani*) of betel vine. Jadeja (2003) observed that fungal antagonists like *Trichoderma* spp. were highly effective for inhibiting mycelial growth and retarding pycnidial formation of *Phomopsis vexans* causing disease in brinjal. *T. koningii* exhibited the maximum antagonistic activity. Bacterial antagonists, e.g. *Bacillus* spp. and *Pseudomonas fluorescens* were also highly effective against the pathogen (Meena *et al.*, 2000).

Ramamoorthy and Samiyappan (2001) suggested that *Pseudomonas flourescens* isolates were effective bacterial antagonist for the management of fruit rot of chilli caused by *Colletotrichum capsici*. Ramamoorthy *et al.* (2002) characterized twenty isolates of fluorescent pseudomonads and evaluated their ability to control damping-off in tomato (*Lycopersicon esculentum*) and hot pepper (*Capsicum annum*). Baruah and Kumar (2002) isolated an antibiotic and siderophore producing *Pseudomonas* strain from virgin soils (with forest trees) which displayed *in vitro* antibiosis against many plant pathogenic fungi.

Monte (2001) reported that antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications have been carried out with different strains of the fungus *Trichoderma* spp. Most of these strains were classified as imperfect fungi since they have no known sexual stage. Some *Trichoderma* species are morphologically similar to the anamorph *Hypocrea*, and their internal transcribed spacer (ITS) sequences have revealed their taxonomic proximity.

Molecular characterization and phylogenetic analysis have allowed strains of *T. harzianum* originally identified as the same species to be assigned to different species clustered into distinct sections and groups. No BCA corresponds to biotypes of *T. harzianum* that is pathogenic to mushrooms (Hermosa *et al.* 2000).

A comparative study of chemical, biological and integrated control of wilt of pigeon pea caused by *Fusarium udum* was done by Pandey and Upadhyay (1999). In chemical control, bavistin was found highly effective, while *Trichoderma viride* and *T. harzianum*-C isolates were found best among biocontrol agents. Integration of biocontrol agents with bavistin was not beneficial. However, integration of the bioagents with thiram reduced wilt incidence significantly. Thus, seed coating with bioagents proved better and safe for the management of wilt of pigeon pea.

Vinas *et al.* (1998) registered the yeast, *Candida oleophilia* for control of postharvest decay on fruit crops. The yeasts, *Cryptococcus infirmo-minutus* and *Candida sake* successfully controlled brown rot and blue mold on sweet cherry and three diseases of apple, respectively.

Swalding and Jeffries (1998) several bacterial microorganisms are being developed for postharvest disease control. For example, strains of *Bacillus pumilus* and *Pseudomonas fluorescens* have been identified that exhibit successful control of *B. cinerea* in field trials of strawberry.

Panday *et al.* (1997) reported that a large number of bacteria and fungi were isolated from the rhizosphere of established tea bushes over a period of one year. Fiftyone of these bacterial isolates were tested for their antifungal activity against 12 test fungi, which include 9 minor and 3 major pathogens of tea. The bacterial isolates exhibiting the highest antifungal activity were also the most dominant bacterial species in the rhizosphere.

Chet *et al.* (1997) several phytopathogenic fungi, such as *Phythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium*, not only attack growing crops but also attack stored fruits. Chemical compounds have been used to control plant diseases (chemical control), but abuse in their employment has favored the development of pathogens resistant to fungicides.

Postmaster *et al.* (1997) reported that biocontrol using antagonistic bacteria has been successful in a number of plant species. For example, bacterial isolates from the surface of banana fruits exhibited an antagonistic ability to suppress anthracnose disease caused by *C. musae*. Other antagonistic bacteria such as *Pseudomonas fluorescens*, *B. subtilis* and *B. megatherium* were shown to control agents of the major cotton diseases, *Xanthomonas campestris* pv. *malvacearum*, *Rhizoctonia solani*, *Fusarium vasinfectum* and *Verticillium dahliae*. Three bacterial strains *B. subtilis*, *B. cereus* and *Pseudomonas corrugata* were shown to control, *Gaeumannomyces graminis* var *tritici* which causes take-all disease, and rhizoctonia root rot caused by *Rhizoctonia solani* on wheat seedlings. Epiphytic bacteria isolated from apples, pears, and the surface of apple leaves showed antagonistic activity against *Penicillium expansum* (blue-mold), while *Botrytis cinerea* (gray-mold) was an effective biocontrol agent on apple fruits.

Janisiewicz and Jeffers (1997) reported that at present, a class of products containing *Pseudomonas syringae* Van Hall, active against the genera *Botrytis*, *Penicillium*, *Mucor* and *Geotrichum*, are available, as well as a product containing *Candida oleophila* Montrocher, effective against *Botrytis* and *Penicillium* spp.

Mari *et al.* (1996) reported that two strains of *Bacillus pumilus* and *Bacillus amyloliquefaciens* were recognized to be effective against *Botrytis cinerea* in pears.

Korsten and De-Jager, (1995) revealed that several mechanisms have been postulated which might play a role in control of post-harvest diseases by *B. subtilis*, e.g. nutrient competition, competitive colonisation and production of an inhibitory substance.

Guizzardi *et al.* (1995) treated fruits by dipping in fungal formulations using Trichodex (*Trichoderma harzianum*) and *Trichoderma pseudokoningii* to control *Monilinia laxa* in stone fruits and *B. cinerea* in apples. Treatments against postharvest fruit diseases resulted in the reduction of disease incidence.

Caccioni *et al.* (1995) reported that hexanal and benzaldehyde, produced by etheric stone fruit metabolism, also has a fungistatic/fungicidal activity when utilized in postharvest treatment against *Monilinia laxa* and *Rhizopus stolonifer*. The use of these substances as antimicrobial agents are interesting field of investigation as toxicity to mammals is quite low, and their degree of volatility allows their use for fumigation in cold storage or for active packaging.

Mishra and Dubey (1994) reported that in different parts of the world, attention has been paid towards exploitation of plant products as novel chemotherapeutants in plant protection. Because of non phytotoxicity, systemicity, easy biodegradability and stimulatory nature of host metabolism, plant products possess the potential to be of value in pest management.

Wilson and Wisniewski (1994) indicated the following characteristics of an ideal antagonist: genetic stability, efficacy at low concentrations and against a wide range of pathogens on various fruit products, simple nutritional requirements, survival in adverse environmental conditions, growth on cheap substrates in fermenters, lack of pathogenicity for the host plant and no production of metabolites potentially toxic to humans, resistance to the most frequently used pesticides and compatibility with other chemical and physical treatments. Yeast seem to possess a good number of the above-

mentioned features and, during the last few years, research has been focused on the selection and study of yeast (Chalutz and Droby, 1998).

Smilanick (1994) reported that antagonists isolated from the surface of plants make them more likely to succeed because of their colonization ability and environmental adaptation (Wilson, C.L. and Wisniewski, M.E. 1989).

Korsten (1993) reported that various bacteria, filamentous fungi and yeasts have been isolated from avocado surfaces using leaf imprint and leaf and fruit washing techniques. From the 106 representative fungal and 176 dominant bacterial isolates, 36 fungi and 48 bacteria were identified to genus or species level. The most promising antagonist *Bacillus subtilis* was selected for large-scale field spray applications at Westfalia Estate and Omega.

Janisiewicz and Marchi (1992) observed that a strain of *Pseudomonas syringae* van Hall was found that controlled both Blue and Gray Mold of pome fruit. It was subsequently registered, and is now sold commercially for postharvest disease control.

Wisniewski and Wilson (1992) reported that a number of microorganisms (bacteria, yeasts and fungi), which effectively control postharvest pathogens, have been identified for postharvest control.

Korsten *et al.* (1991) reported that *Bacillus subtilis* was evaluated under commercial packinghouse conditions for the control of postharvest fruit diseases of naturally infected Hass fruit. *B. subtilis*, applied in commercial Tag wax at various concentrations as well as in a water dip, significantly reduced anthracnose, *Dothiorella/Colletotrichum* fruit rot complex and stem-end rot. The antagonist water dip treatment was also equally or more effective than prochloraz water dip in controlling the postharvest diseases.

McLaughlin *et al.* (1990) reported that three yeast strains (strains US-7, 82, and 101) have biological control activity against various postharvest fungal pathogens of fruits and vegetables, including *Penicillium* rots of apples and citrus and *Botrytis* rot of apples. In his reports the researchers have described those strains as *Debaryomyces hansenii* (anamorph, *Candida famata*) or *Candida* sp. strains. They performed additional physiological, DNA reassociation, and mannan characterization tests that clearly established a new taxonomic classification for those strains, *Candida*

guilliermondii. They also proposed amendment of the physiological test profile in the taxonomic description of *C. guilliermondii*.

Droby *et al.* (1989) reported that the use of yeasts as antagonists appears to be quite promising, although the mechanism was not fully elucidated. However, there are indications that competition for nutrients and space is involved, along with direct parasitism as for the US-7 strain of *Pichia guilliermondii*, antagonist of *P. digitatum* in grapefruit, and of *B. cinerea* in apples. In addition to the ability of yeast cells to grow very quickly (and thus to remove nutrients and space from the pathogen), they are able to produce hydrolytic enzymes (β 1-3 glucanase) capable of attacking the cell walls of *B. cinerea*, and extracellular polymers that appear to have antifungal activity (Droby *et al.*, 1993).

Wilson and Wisniewski, (1989) have described control of post-harvest fungal pathogens by bacteria and yeast antagonists (such as *Bacillus subtilis*, *Pseudomonas cepacia*, *Pseudomonas syringae*, *Enterobacter aerogenes*, *Enterobacter cloacae* and *Debaryomyces hansenii*) for a variety of stored vegetables and fruit including apple, apricot, cherry, citrus, grape, nectarine, peach, pear, pepper, persimmon, plum, potato and tomato.

Pusey (1989) reported that several biological control agents have been developed in recent years, and a few have actually been registered for use on fruit crops. The first biological control agent developed for postharvest use was a strain of *Bacillus subtilis*. It controlled peach brown rot.

Korsten *et al.* (1989) reported that in South Africa biocontrol is a new approach to avocado postharvest disease control. The biological approach was evaluated against standard chemical control measures on a pre-and postharvest level. Antagonists *Bacillus lichineformis* and *B. subtilis* effectively reduced stem-end rot, anthracnose and the *Dothiorella/Colletotrichum* fruit rot complex, when applied as field preharvest sprays, as well as packhouse postharvest dip treatments.

Chitosan's compatibility with several antagonistic yeasts also appears to be appreciable, although its feasibility as an antifungal substance has yet to be confirmed in terms of its safety for human consumption and its effect on the organoleptic quality of fruits and vegetables (Wilson and Wisniewski, 1994).

The antagonistic effect of *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* was well established as reported by several workers. Wu *et al.*, (1986) and Vinod (1988) reported the antagonistic effect of *A.niger*. The antagonistic property of *A. flavus* against many pathogenic microorganisms has been reported by Massoor and Chandra (1987) and Deb (1990) though not specific against *Pythium* spp. The hyphal coiling and production of inhibitory substances by different species of *Trichoderma*, resulting in dieback and disintegration of *Pythium* spp were reported by Raju (1991) and Vinod *et al.* (1991). Mukherjee and Sen (1992) observed that the culture filtrate of *A. fumigatus* inhibited the growth of *Macrophomina phaseolina* and sclerotial germination.

Postharvest disease control by botanicals

Plants have, and continue to be, sources of antifungal agents (Hostettmann *et al.*, 2000). Many plant species contain antifungal compounds. To develop commercial products, a large quantity of the species has to be cultivated, raising an additional level of complication. If invasive and weedy species contain good antifungal activity they may be a useful source of antifungal compounds or extracts because large quantities of material are available.

The traditional practice of using plant preparations to combat fungal infection has gained attention and currently the focus is on detection of new antifungal components from plants that have no negative effect on the environment or on animal and human systems. Many higher plants produce important organic compounds, pharmaceuticals and pesticides. Hamburger and Hostettmamnn (1991) reported that total number of plant chemicals may exceed 400,000 of which more than 10,000 are secondary metabolite whose major role in plant is defensive in nature. Thus plant based secondary metabolites, which have defensive role, may be exploited for the management of plant pathogens. However most species of higher plants have never been described, much less surveyed for chemicals or biological active constituents and new sources of commercially valuable pesticides remains to be discovered (Balandrin *et al.*, 1985)

Plants in nature synthesize numerous carbon compounds, basically for their physiological functions or for use as chemical weapons against pathogens, insects and predators (Fatope, 1995). It has been expected that 70-80% of total world population

largely depends on traditional herbal medicine to meet their primary health care need (Hamayun *et al.*, 2006). Plants have been proved as useful source of quite a lot of antifungal molecules that are harmless and benevolent to the environment. There are definite advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Moreover, repeated application of fungicides to attain advantageous level of disease control has been discouraged by some of the farmers (Singh and Sinha, 2005).]

Reddy *et al.* (2007) reported the antifungal component of cloves. They isolated, characterized and tested the efficacy of cloves against *Aspergillus* spp. The major component, eugenol was identified on TLC plate as dark coloured spot with R_f 0.5 along with standard. In TLC plate bioautography test, TLC plates were spray inoculated with four species of *Aspergillus* (*A. flavus*, *A. paraciticus*, *A.niger*, *A. ochraceus*) and eugenol on TLC plates inhibited mycelia growth of all four species of *Aspergillus*.

Meena *et al.* (2007) evaluated antibacterial activity of seven semi purified plant extracts made from flowers, leaves, fruits, stems, pods and seeds of some plants and four antimicrobial chemicals. The bacterial plant pathogens were *Pseudomonas solanacearum*, *Xanthomonas campestris* pv. *Campestris*, *Xaxonopodis* and *Xanthomonas* pv.*Citri*. Evaluation was done by disc diffusion technique. Product componantes from mahua flowers and Satyanashi leaves were found effective against *Pseudomonas solanacearum* at 1000 ppm.

Mewari *et al.* (2007) screened two mosses viz. *Entodon plicatus* C. Muell and *Rhynchostegium vagans* jaeg for their antimicrobial activity against *Bipolaris sorokiniana* (Sacc. and Sorok), *Fusarium solani* (Mart.) Sacc.(fungi) and *Pseudomonas sclanacearum*, *Xanthomonas oryzae* pv.*oryzae* (bacteria). Aquous extracts of the two mosses were found to be ineffective. Ethanolic extracts of *E. plicatus* showed maximum inhibition (42%) of *B.Sporokiniana* and petroleum ether extract of *R.vagans* exhibited max. inhibition (45%) of *B.Sporokiniana*. Extract of *R. vagans* were found to be more effective inhibitors of *F. solani* than those of *E. plicatus*. Ethanolic extract of *R.vagans* showed maximum inhibition (44%) of *F. solani* whereas alcoholic extracts of both the mosses showed more effective antimicrobial activity.

Malabadi and vijay kumar (2007) evaluated the antifungal activities of acetone, hexane, dichloromethane and methanol extracts of leaves of four plant species (*Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* and *Catunaregum spinosa*) against pathogen viz. *Candida albicans*, *Kluyveromyces polysporus*, *Aspergillus niger*, *Aspergillus fumigatus*. High antifungal activity was observed with methanolic extract of *Anaphylis wightiana* against all the test pathogens with the MIC values ranging from 0.02 to 0.06. Methanolic extract of *Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* have very strong antifungal activity against tested pathogens particularly *C. albicans* and *K. polysporus*.

Guleria and Kumar (2006) searched for bioactive compounds from lipophilic leaf extracts of medicinal plants used by Himalayan people. They screened antifungal properties by direct bioautography. *Alternaria alternata* and *Curvularia lunata* were used as test organism in bioautography. The results were evaluated by the diameter of the fungal growth. They showed five effective plant species with antifungal activity among the 12 investigated. They used CHCl_3 : CH_3OH (1:9, v/v) as a solvent to develop silica gel TLC plates. Clear inhibition zones were observed for lipophilic extracts of *Vitex negundo* (RF value 0.85), *Zantoxylum alatum* (RF value 0.86), *Ipomea carnea* (RF value 0.86), *Thuja orientalis* (RF value 0.80) and *Cinnamomum camphora* (RF value 0.89). The best antifungal activity was shown by lipophilic leaf extract of *T. orientalis*.

Kiran *et al.* (2006) screened thirty plant extracts (aqueous extract) against the pathogen *Sclerotium rolfsii* *in vitro* to examine the inhibitory effect on mycelial growth and sclerotial production. Maximum inhibition (74%) of mycelial growth was recorded at 10% concentration of plant extract (*Prosopis juliflora*). Other two antifungal plant extracts were from *Agave Americana* (showed 68% overall inhibition) and *Nerium indicum* (showed 54% overall inhibition). The inhibition (94%) of sclerotial production was exhibited by *Agave americana* and almost similar inhibition was shown by *Clerodendron inerme* leaf extracts. Leaf extract of *Riccinus communis* and fruit extract of *Riccinus communis* also gave well results (showed 72%) inhibition.

AL-Howiriny *et al.* (2005) reported three new diterpenes and biological activity of different extracts of *Jasonia monata*.

Kuiate *et al.* (2005) studied about the chemical composition and *in vitro* anti fungal properties of essential oils from leaves and flower of *Erigeron floribundus*.

Antifungal activity of selected species of *Terminalia* and *Curcuma* has been studied by Fyhrquist *et al.*, (2004). Saxena *et al.* (2003) and Flach *et al.* (2002) showed anti-fungal activity of some plants and also analyzed the antifungal properties chemically. Anti-fungal action of essential oil and other components have been reported by several scientists (Deena and Tophil, 2000, Demirci *et al.*, 2000 Mathpal *et al.*, 2002 and Simic *et al.*, 2004). Anti-fungal activity of *Tagetes patula* extracts have been shown by Mares *et al.* (2002). Anti-fungal sesquiterpenes from stem bark of *Guarea macrophylla* has also been demonstrated by Logo *et al.*, (2002)

Deepak *et al.* (2005) used methanolic extracts of forty plant species commonly growing across India and screened for antispore activity against *Sclerospora graminicola*, the causative organism of pearl millet downy mildew. The methanolic extracts of nine species did not show any effect, whereas the activity of the extracts of *Clematis gouriana*, *Evolvulus alsinoides*, *Mimusops elengi*, *Allium sativum* and *Piper nigrum* were commensurable to that of the marketed botanical fungicides. The extracts of 11 species (*Agave americana*, *Artemisia pallens*, *Citrus sinensis*, *Dalbergia latifolia*, *Helianthus annuus*, *Murraya koenigii*, *Ocimum basilicum*, *Parthenium hysterophorus*, *Tagetes erecta*, *Thuja occidentalis* and *Zingiber officinale*) exhibited remarkable antispore effect even after 10-fold dilution of the crude extracts. But in the case of remaining 15 plants the crude extracts lost activity after 10-fold dilution. The antispore activity of commercialised *Azadirachta* preparation (Nutri-Neem) was more pronounced than that of Reynutria based on (Milsana) and Sabadilla (veratrin).

Curtis *et al.* (2004) reported that garlic extract showed activity against the plant pathogenic bacteria *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas syringae* pv. *maculicola*, *P.s.* pv. *phaseolicola*, *P.s.* pv. *tomato*, *Xanthomonas campestris* pv. *campestris*, the fungi *Alternaria brassisicola*, *Botrytis cinerea*, *Plectosphaerella cucumerina*, *Magnaporthe grisea*, and the oomycete *Phytophthora infestans*.

Fukai *et al.* (2003) found that antifungal agent from the roots of *Cudrania cochinchinensis* against *Candida* and *Aspergillus* sp.

Shin and Kang (2001) reported some antifungal activities of essential oils from cultivars of *Brassica juncea*. Ogunwande *et al.* (2001) analysed methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospermum pradoxum* (*Vitellaria paradoxa*) and revealed the presence of alkaloids (in leaves and stem barks), flavones (in stem and root bark), saponins (in root bark), steroids (in stem bark, fruits and seed kernels) and tannins (in leaves and root bark) which have antimicrobial activity against bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Ralstonia solanacearum* and *Bacillus cereus*) and fungi (*Fusarium oxysporum* and *Candida albicans*).

The leaves of five *Betula* species, *B. pendula*, *B. browicziana*, *B. medwediewii*, *B. litwinowii* and *B. recurvata* collected from different parts of Turkey were hydrodistilled to yield the consequent essential oils. The essential oils showed antifungal activity against various phytopathogenic fungi like *Cephalosporium aphidicola*, *Drechslera sorokiniana*, *Fusarium solani* and *Rhizoctonia cereals* (Demirci *et al.*, 2000).

Botanicals extracted from many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties (Satish *et al.*, 1999; Okigho and Ogbonnay, 2006; Sharift *et al.*, 2006; Bouamama *et al.*, 2006; Ergene *et al.*, 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and are less dangerous to consume in contrast to synthetic pesticides (Varma and Dubey, 1999). Several authors have reported antifungal activity of plant extracts against pathogens of rice, tomato, wheat, pea and other important crops (Rana *et al.*, 1999; Sindhan *et al.*, 1999; Hu *et al.*, 2001). However such report involving tea pathogens are very few (Chakraborty *et al.*, 1991, Saha *et al.* 2005a, b). Exploitation of naturally available chemicals from plants, which retard the reproduction of undesirable microorganism, would be more realistic and ecologically sound method for protection of tea plants against diseases. This should have a prominent role in development of future commercial fungicides for crop protection strategies with special references to the management of foliar diseases (Varma and Dubey, 1999; Gottlieb *et al.*, 2002)

The hexane and methanol extracts of sixteen plants of the family Caesalpinaceae, collected around Karachi, Pakistan, were phytochemically screened and tested for their

antibacterial and antimicrobial activity. As compared to hexane extracts, the methanol extracts of all the examined plants showed stronger growth inhibitions against both bacteria and fungi, *Cassia* species being the biologically more active plants (Ali *et al.*, 1999). Carpinella *et al.* (1999) reported that the ethanol extract of *Melia azedarach* ripe fruits showed fungistatic (MIC 50-300mg/ml) and fungicidal (MFC60-500mg/ml) activity against *Aspergillus flavus*, *Fusarium moniliforme*, *Microsporum canis* and *Candida albicans*

The antimicrobial activities of *Valex* (the extract of valonia), the extracts of mimosa bark, gullnut powders, *Salvia ancheri* Benthum. var. *ancheri* and *Phlomis bourgei* Boiss. were studied. The results of the study indicated that mimosa bark extracts had the greatest antibacterial activity, followed by the *Valex*, gullnut powders, *Salvia ancheri* var. *ancheri* and *Phlomis bourgei* extracts, respectively. Furthermore, it was found that gullnut powders and the extracts of mimosa bark contained high amounts of tannins and showed antifungal activity (Digrak *et al.*, 1999).

Two hundred and four species of traditional Chinese herbal medicines belonging to 80 families were collected from Yunnan Province in People's Republic of China and tested for antifungal activities using a *Pyricularia oryzae* bioassay. Twenty-six herbal medicines from 23 families were active against *P.oryzae* and the ethanol extract of *Dioscorea camposita* (dioscoreaceae) exhibited the most bioactivity among all the tested samples (Hu *et al.*, 1999).

The antibacterial activity of ethanol extracts of 15 plant species used in the traditional medicine in Jordan and other Middle East countries were tested. Extracts of certain parts of these plants were tested *in vitro* against 14 pathogenic bacterial species and strains using the agar diffusion methods. Three plants exhibited broad spectrum antibacterial activity: *Punica Granatum* L., *Quercus infectoria*, and *Rhus coriaria* L. The most susceptible bacteria were *Pseudomonas aeruginosa*, *Bacillus cereus* and *Streptococcus pyogenes* (ATCC 12351), and the most resistant species were *Escherichia coli* (ATCC 25922 and clinical isolates), *Klebsiella pneumoniae*, *Shigella dysenteriae* (ATCC 49345), and *Yersinia enterocolitica* (ATCC 9610) (Nimri *et al.*, 1999).

Bianchi *et al.* (1997) tested *Fusarium solani*, *Colletotrichum lindemuthianum*, *Pythium ultimum* and *Rhizoctonia solani* and found that garlic extracts inhibited mycelial

development *in vitro*. Ali *et al.* (1999) screened hexane and methanol extracts of sixteen plants of the family Caesalpiniaceae, collected around Karachi, Pakistan and were tested for their antibacterial and antimicrobial activity. As compared to hexane extracts, the methanol extracts of all the examined plants showed stronger growth inhibition against bacteria and fungi, *Cassia* species being the biologically more active plant. Ethanol extracts of *Melia azadirachta* fruit showed fungistatic (MIC 50-300 mg/ml) and fungicidal (MFC60-500 mg/ml) activity against *Aspergillus flavus*, *Fusarium moniliforme*, *Microsporium canis* and *Candida albicans* (Carpinella *et al.*, 1999).

Verastegui *et al.* (1996) investigated the antifungal activity of several widely distributed plants in the vegetation on north Mexico and the southern USA. The molds were evaluated on the growth of yeasts and moulds: *Candida Krusei*, *Cnadida rugosa*, *Cryrococcus neoformans*, *Cryptococcus albidus*, *Microsporium canis*, *Microsporium gypseum*, *Trichophyton tonsurans*, *Epidermophyton flocosum* and *Sporotrix Schenckii*. The extracts showed a good antifungal activity against the microorganism.