CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Citrus (*Citrus sinesis* L.) is one of the most important fruit crops known by humans since antiquity and is a good source of vitamin "C" with high antioxidant potential (Gorinstein *et al*., 2001). Citrus originated from south-eastern Asia, China and the east of Indian Archipelago from at least 2000 BC (Swingle, 1943; Webber *et al*., 1967; Gmitter and Hu, 1990). The fruit has been introduced to the new world *via* the great trade routes of Africa to the eastern Mediterranean basin by the Arab traders while the crusaders brought the fruit to Italy, Spain and Portugal around 1000 AD (Scora, 1975). The fruit was introduced further to the western hemisphere by Columbus on his second voyage in 1493 (Samson, 1980) and the planting material to the Cape in South Africa by a Dutch merchant in 1654 (Oberholzer, 1969). Currently, citrus is cultivated in the subtropical and tropical regions of the world between 40^o north and south latitude in over 137 countries on six continents and generates about 105 billion US dollar per year in the world fruit market (Ismail and Zhang, 2004). In Ethiopia, although the introduction, production and consumption of citrus as a horticultural crop is very recent (Seifu, 2003), the current production and area coverage has increased through private, association and government firms to meet the local and export demands.

As with other fruits, citrus is attacked by several pre- and/or postharvest pathogens that affect fruit quality. Green and blue mould infections caused by *Penicillium* spp. (Droby *et al*., 1989), anthracnose caused by *Colletotrichum gloeosporioides* Penz (Whiteside *et al*., 1988; Davies and Albrigo, 1994), and sour rot caused by *Geotrichum candidum* Link ex Pers (Howard, 1936; Whiteside *et al*., 1988; Chalutz and Wilson, 1990) are some of the major postharvest problems that cause market losses. In developing countries, where protection and proper handling of fresh fruit is inadequate, losses during transit and storage are even greater mounting up to about 50% of the harvested crop (Wisniewski and Wilson, 1992). In Ethiopia, although there are not much comprehensive data available, estimates by Eyob (1997) showed that more than 50% of the fresh fruit produced is lost postharvestly.

Currently, to minimize losses caused by citrus fruit pathogens, synthetic chemicals are applied either pre- or postharvestly. However, the application of synthetic chemicals to control postharvest diseases often result in chemical residues on food that may affect human health (Norman, 1988). In addition, the development of chemical resistant strains may result in reduced efficacy of synthetic chemicals (Janisiewicz, 1987; Wilson and Wisniewski, 1989).

Development and use of alternative postharvest control options involving biological agents are critically important (Conway *et al*., 1999; El-Ghaouth *et al*., 2000; Korsten *et al*., 2000; Janisiewicz and Korsten, 2002). Moreover, natural plant extracts may provide an environmentally safer, cheaper and more acceptable disease control approach (Kubo and Nakanishi, 1979; Dixit *et al*., 1995; Wilson *et al*., 1997).

This chapter briefly reviews postharvest diseases generally, with particular emphasis on those important in Ethiopia. Non-chemical control options that have been studied so far and/or are currently in use are also reviewed. The possible mode of action of biopesticides is also reported. The future use of biocontrol agents from an Ethiopian perspective is also discussed.

2.2 World citrus production, consumption and marketing

Of the total world citrus production, sweet orange (*C. sinensis*) constitute the most important proportion accounting for more than two thirds of global area coverage (FAO, 2004). Currently, ten species of edible citrus are known of which eight are commercially cultivated and five are of great economic importance (Salunkhe and Desai, 1984). Annually, more than 104 million tons of citrus are produced and about 15 million tons are traded (FAO, 2004). In Africa, the total surface area under citrus production is 1.3 million hectares, of which, 44 000 ha is in South Africa and 4 500 ha in Ethiopia (Table 2.1). Despite its recent introduction to Ethiopia (Seifu, 2003), citrus farming is scattered throughout the country (Lipsky, 1962; FAO, 1965).

2.3 Citrus fruit diseases

Postharvest losses and decay of citrus fruits can be traced to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage activities. Preharvest infections are mainly caused by fungal pathogens such as *Phytophthora* spp. *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz, *Botrytis cinerea* Pers ex Fr, *Diplodia natalensis* Pole-Evans, *Phomopsis citri* Faw, and *Alternaria citri* Ellis and Pierce (Browning *et al*., 1995; El-Ghaouth *et al*., 2002).

Table 2.1 **A comparison of Ethiopian and South African citrus production area and volumes compared to the rest of the continent and the world for the period 1985-2004**

Country	Total area harvest (ha)	(%) Growth of total area harvest	Total production Mt/year	(%) Growth of production	Reference
World	4 908 106 – 7 090 356	30.8	64 053 474 –103 685 840	37.6	FAO, 2004
Africa	1 009 277 – 1 325 135	23.84	6 821 085 – 11 088 509	38.5	″ ″
South Africa	35 400 – 69 200	48.8	706 228 – 1 712 149	58.75	″ ″
Ethiopia	3 115 – 4 800	35.1	23 600 – 29 800	20.8	CACCE, 2003; FAO, 2004

Legend: CACCE = Central Agricultural Census Commission of Ethiopia.
FAO = Food and Agriculture Organization.

Stem-end fruit infections caused by *Diplodia*, *Phomopsis*, and *Alternaria* spp. remain quiescent until the fruit becomes senescent during prolonged storage (Salunkhe and Desai, 1984; El-Ghaouth *et al*., 2002). Infections initiated by *Phytophthora* spp. occur during wet periods before harvest, while *B. cinerea* infections can occur in the orchard and during storage (Batta, 2004). On the other hand, postharvest infections that occur through surface wounds inflicted during harvest and subsequent handling are mainly caused by pathogens such as *Penicillium digitatum* Sacc, *Penicillium italicum* Wehmer, *Geotrichum citri-aurantii* (*syn. G. candidum* Link ex Pers), and *Trichoderma viride*.

Among the wound pathogens, green mould (*P. digitatum*) and blue mould (*P. italicum*) account for most of the decay of citrus fruit worldwide (Plaza *et al*., 2003). Sour rot caused by *G. citri-aurantii* is the most rapidly spreading postharvest disease and can be severe on fruit stored at temperatures above 10 °C (El-Ghaouth *et al*., 2002). Some diseases such as algal disease (algal climb), canker and insect damage caused by thrips, which cause superficial (rind blemish) problems, do not affect yield or juice quality but may affect market appeal (Whiteside *et al*., 1993). In addition to these, fruit infections triggered by insect, mite and fungal attacks could be more intense and difficult to control in humid lowland areas of the tropics (Samson, 1980). Worldwide, postharvest losses of fruits and vegetables have been estimated to be 25% (Wisniewski and Wilson, 1992). In developing countries, where protection and proper handling of fruit is lacking or minimal, the losses can be as high as 50% (Coursey and Booth, 1972). In Ethiopia, such an estimate is considered conservative (Eyob, 1997). However, a higher percentage of what could be expected because of poor handling practices, lack of cool storage facilities and insufficient postharvest treatments (Eyob, 1997). A summary of the major citrus postharvest diseases, causal agent, infection type and site and spread of citrus disease infection is depicted in appendix I table 1).

2.3.1 Major citrus postharvest diseases epidemiology and control

2.3.1.1 Green mould

Over 99 species of *Penicillium* have been described (Carlos, 1982). Conidia of *P. digitatum*, the causal agent of green mould, are produced in chains and may vary in size (4 -7 x 6 – 8 μm) and shape (Fig. 2.1a and b) (Carlos, 1982). Colonies on artificial media are similar in appearance to the mould that develops on infected fruit.

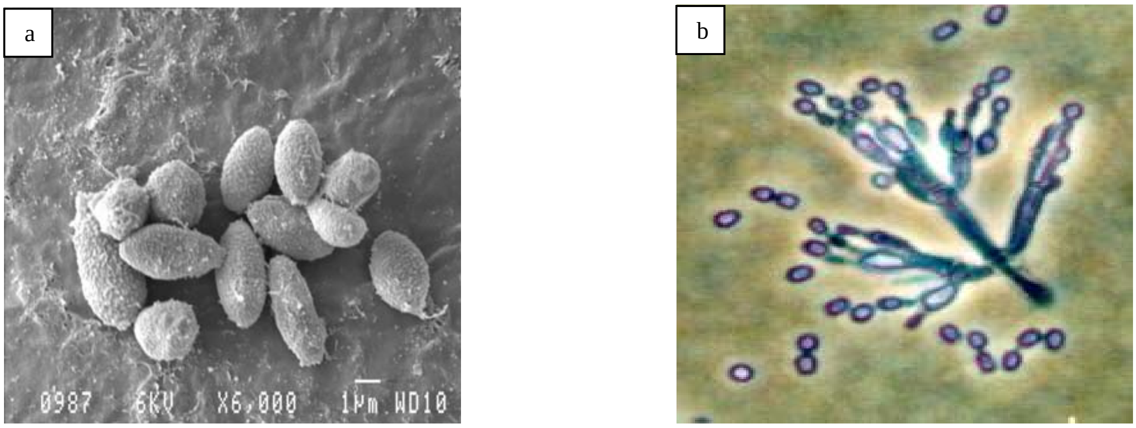

Fig. 2.1.Reproductive structures of *Penicillium digitatum,* a) Spores, (b) Conidiophore bearing spores producing phialides (Courtesy: Morgan, 2006).

2.3.1.1.1 Symptoms

Moisture plays an important role in enhancing spore growth and development. The initial symptom of green mould appears as a soft, watery, slightly discoloured spot with 6 – 12 mm diameter initially similar to sour rot and blue mould infections (Brown, 1973). Spores from the surface of infected fruit, air, field, packing area, storage room, transport containers and market places are the source of infection. The lesion diameter enlarges to 2-4 cm within 24-36 h at room temperature and the decay soon involves the juice vesicles. In five to six days, olive green spores are produced following the appearance of white mycelium around the rind encompassing the entire fruit (Fig.2.2). (http://www.sardi.sa.gov.au/pages/horticulture/citrus/hort_citp_postpacksanitation.htm)

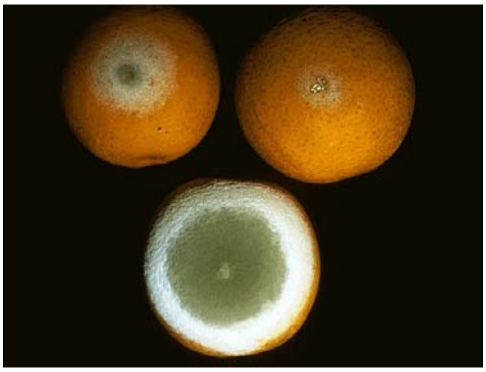

Fig. 2.2. Citrus green mould on fruit

2.3.1.1.2 Disease cycle and epidemiology

Green mould survives in the orchard from season to season primarily as conidia. Infection is initiated by airborne spores, which enter the rind through mechanical injuries (Kuramoto, 1979). Nutritionally, the pathogen is a necrotroph, which require nutrients only for germination around the wound site (Janisiewicz *et al*., 2000). A minor injury to the oil glands during harvesting and transportation promotes infection (Brown, 1973). In packed containers, the fungus doesn't usually spread from decayed fruit to adjacent intact healthy fruit. Instead, the infection and sporulation cycle can be repeated many times through the season in a packinghouses and inoculum pressure increases as the picking season advances, if precautions are not taken (Janisiewicz and Korsten, 2002). Contamination spread when spores detach from diseased fruit during the opening of packing cartons. Green mould develops most rapidly at temperatures near 24 °C and more slowly above 30 °C and below 10 °C. Rotting is almost completely inhibited at freezing temperature (0-1 °C) (Plaza *et al*., 2004).

2.3.1.2 Sour rot

Endomyces geotrichum Butler and Petersen (anamorph, *Geothricum candidum* Link ex Pers.) the causal agent for sour rot presents some conidia of 2 - 8 x 3 - 50 µm diameter (Fig. 2.3a). The fungus grows rapidly on potato dextrose agar, producing a dull gray-white colony with chains of arthrospores (Fig. 2.3b) (Butler and Eckert, 1962).

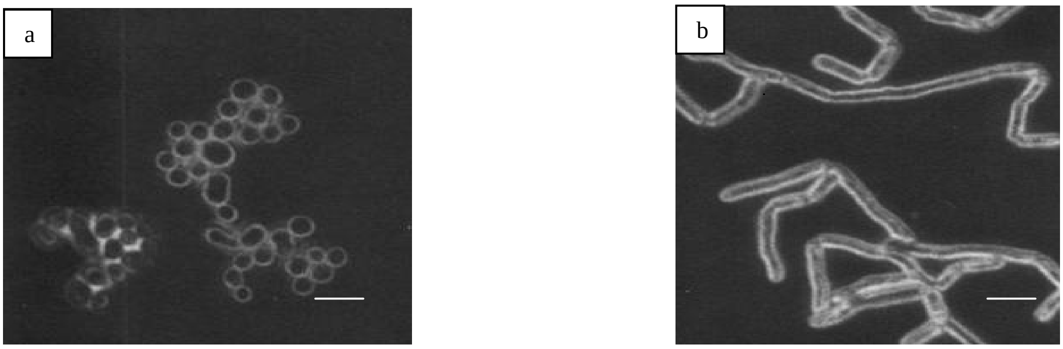

Fig. 2. 3. Reproductive structure of *Geotrichum candidum*, a) Conidia and b) Chains of arthrospores appearing dull gray white colony (bar = 1µm).

2.3.1.2.1 Symptoms

Citrus sour rot infection has the most unpleasant smell of all decays known. The initial symptoms of sour rot infections are similar to those of green and blue moulds. The cuticle is more susceptible to handle as compared to the lesions formed by *Penicillium*-induced moulds (Sommer and Ewards, 1992). The fungus degrades the rind, segment walls, and juice vesicles into a slimy, watery mass. At high relative humidity, the lesions may be covered with a yeasty, sometimes wrinkled layer of white or cream-coloured mycelium (Baudoin and Eckert, 1982) (Fig.2.4).

Fig. 2. 4. Sour rot infection caused by *Geotrichum candidum*.

2.3.1.2.2 Disease cycle and epidemiology

The pathogen occurs commonly in soils and is windborne or splash borne to surfaces of fruit within the tree canopy. As fruits mature, they become more susceptible to sour rot infection (Baudoin and Eckert, 1982). Disease development depends on high humidity and temperature above 10 ºC, with the optimum range being 25-30 °C. Spores-laden watery debris from infected fruits and orchard soils may contaminate dip thanks, drenchers, washer brushes, belts and spread to other fruits on the packing line. Upon infection, the sour odour associated with the advanced stages of sour rot attracts flies (*Drosophila* spp.), which can disseminate the fungus and cause other injured fruit to become infected.

2.3.1.3 Brown Rot

Phytophthora spp. [*Phythophthora nicotianae* Van Breda de Hann (syn. =*P. parasitica* Dast.)] is the causative agent of citrus brown rot, which develops mainly on fruits growing near the ground (Timmer and Menge, 1988).

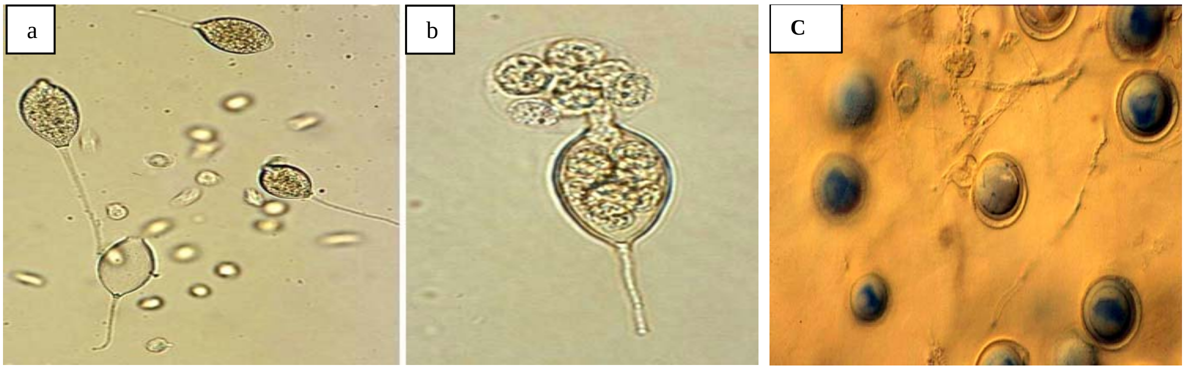

Fig. 2.5. Sporangia and zoospores of *Phytophthora* spp. a) Sporangia and zoospores b) a sporangium releasing zoospores, and c) Oospores of *Phytophthora* spp. in a culture plate (Courtesy: Babadoost, 2006).

2.3.1.3.1 Symptoms

Phytophthora infection may cause various disease symptoms on the mature fruit, trunk and root of citrus trees. Infection on fruit starts when *Phythophthora* spores from the soil splash onto the tree during rainstorms and infections develop under continual wet conditions. Initially, the firm, leathery lesions have a water-soaked appearance, but they soon turn soft and have a tan to olive brown colour and pungent odour (Fig.2.6). On the tree trunk and roots, shelling and scaling of the bark and development of lesions and gumming are common symptoms.

Fig. 2.6. *Phytophthora* spp. infection (Brown rot) on fruit (Courtesy: Futch and Timmer, 2001).

2.3.1.3.2 Disease cycle and epidemiology

Phytophthora spp. are present in almost all citrus orchards (Ann *et al.*, 2004). Under moist conditions, the fungi produce large numbers of zoospores, which are splashed by rain or irrigation water onto the tree trunks, and low hanging fruits. The pathogen then develops rapidly under moist, cool conditions. On fruit, the infection progresses over the surface, but not beyond the albedo (Fig.2.6). Infected fruits in the early stage of disease development may go unnoticed at harvest and infect other fruit during storage.

2.3.2 Postharvest citrus disease control

Fungicides are commonly applied as field sprays to control fruit diseases and cold chain management practices applied to prevent and/or control quiescent fungal infections of fruits. Despite the use of fungicides, the losses of up to 20% of the harvested product are still recorded in countries even with advanced cold storage facilities (Cappellini and Ceponis, 1984). In developing countries, where the disease management practices and proper handling of postharvest commodities are poor, postharvest losses of fruits and vegetables are rated to about 50% (Eckert and Ogawa, 1985). To minimize losses and improve the shelf life of fruits and vegetables, the application of good pre- and postharvest practices including sanitation, careful harvesting and effective cold chain management practices are crucial.

2.3.2.1 Chemical control

Currently, 23 million kg of fungicides are applied annually to protect crops against diseases and pests throughout the world. Of this, about 26% of crop protectants are used in Europe, North and South America, Oceania and Asia (Tripathi and Dubey, 2004), while Africa constitutes the rest of chemical marketing and use. The application and marketing of fungicides in the USA have been reduced by 1.3% and 6% respectively (Tripathi and Dubey, 2004).

The perception that pesticides are harmful to human health and the environment has lead to the implementation of more restrictive legislation dealing with allowable chemicals and residue levels. Other problems associated with excessive use of pesticides are the development of resistant strains to tiabendazole (Timmer and Duncan, 1999), imazalil (Bus *et al*., 1991; Eckert *et al*., 1994; Timmer and Duncan, 1999) and benomyl (Bus *et al*., 1991). In addition to these, an ecological shift or imbalance in microbial populations is often the result of continuous pesticide use (Reimann and Deising, 2000). The major groups of commercial pesticides, their use and reported pathogen resistance development are summarized in appendix 1 table 2.

2.3.2.2 Non-chemical disease control strategies

The development of alternative postharvest disease control options using either microbial agents (Conway *et al*., 1999; El-Ghaouth *et al*., 2000; Korsten *et al*., 2000; Janisiewicz and Korsten, 2002; Pang *et al*., 2002; Ismail and Zhang, 2004) or natural plant products (Kubo and Nakanishi, 1979; Dixit *et al*., 1995; Wilson *et al*., 1997; Obagwu and Korsten, 2003) have become more important as successful commercial applications have gained ground. Biopesticides (microbial agents and natural plant materials) have the potential to be more environmentally safe and more acceptable by the general public for human use.

2.3.2.2.1 Cultural and physical requirements

Cultural and physical activities represent non-chemical strategies that require manipulation of the environment to decrease disease pressure. In citrus field management systems, soil drainage improvement, use of ridges (to allow air movement and draining in the juvenile phase of crop growth), use of block-raising techniques for better spacing and removal of the inoculum sources are amongst the most prominent practices involved in cultivation of citrus (Dixon, 1984).

At fruit harvesting, maximum care is required to prevent punctures, bruises, and abrasions on fruit rind. Harvesting by clipping reduces the possibility of inflicting wounds as compared to pulling (Claypool, 1983). Citrus fruit subjected to dehydration at low relative humidity after harvest is prone to stem-end rind breakdown, a physiological injury which can predispose fruit to decay (Wardowski and Brown, 2001). Therefore, temperature and humidity management in the postharvest arena is crucial to avoid deterioration of produce and the initiation of infection. The relative humidity (RH) of fruits kept in pallet boxes should be between 90% to 98%, whereas in fibreboard cartons between 85-90% to prevent carton deterioration (Wardowski and Brown, 2001). Effective sanitation practices during pre- and postharvest handling can greatly reduce the incidence of decay. Separation of sound fruits from the decayed ones in storage or distribution or repack centres reduces possible sources of inoculum and prevents contamination (Wardowski and Brown, 2001).

2.3.2.2.2 Bio-pesticides

Bio-pesticides are the new generation crop protectants based on naturally occurring microbial communities on plant surfaces and use of extracts from plant materials.

Microbial pesticides are antagonistic microorganisms, which are screened and developed for their antipathogenic activity. Antagonistic microorganisms can be collected from several sources such as dead arthropods, disease suppressive soils, and healthy plants in epidemic areas. However, epiphytic microflora derived from the commodity to be protected is the most adequate candidates (Wilson and Wisniewski, 1989). In various ways, viruses, bacteria, fungi and micro-fauna have all been observed to give some level of disease control. However, the greatest interest is directed at the use of bacteria and fungi to control soil borne, leaf and fruit diseases (Whipps and McQuilken, 1993). These probably may be attributed to the easy manipulation of the microbial strains as required.

Several species of bacteria and yeasts have been reported to reduce fungal decay of pome fruits (Janisiewicz, 1985; Mercier and Wilson, 1994; Janisiewicz *et al.*, 2000), apple (Janisiewicz, 1988; Roberts, 1990; Vero *et al.*, 2002; Spadaro *et al.*, 2002; Batta, 2004), grape fruit (Droby *et al.*, 2002), avocado (Korsten and De-Jager, 1995; Demoz and Korsten, 2006), pear (Zhang *et al.*, 2005) and mango (Korsten *et al.*, 1991; Koomen and Jeffries, 1993; Govender and Korsten, 2006). Currently, several antagonists have been registered in South Africa for control of postharvest diseases of avocado such as *Bacillus subtilis* (Avogreen) and pome fruit *Cryptococcus albidus* (YieldPlus) (Janisiewicz and Korsten, 2002). Other commercial products such as *Pseudomonas syringae* (BioSave 110 and 111) to control *Geothricum candidum* and *Candida oleophila* (Aspire ^{TM}) to control penicillium on citrus and pome fruit have been registered by Ecogen Inc. in the USA (Shachnal *et al*., 1996). Biopesticides currently registered for commercial use are summarized in appendix 1 table 3.

In citrus, several bacteria such as *Bacillus* spp. have been reported to reduce postharvest decay (Huang *et al*., 1992; Obagwu and Korsten, 2003). The citrus phylloplane contains a complex and diverse population of microorganisms adapted to survive by competition. The use of such organisms could provide alternatives to the use of fungicides (Janisiewicz and Korsten, 2002).

The disease control mechanisms of biopesticides include multiple modes of actions [production of antibiotics (Fravel, 1988), induction host resistance (Droby *et al*., 2002; Poppe *et al*., 2003), synthesis of phytoalexins and/or the accumulation of an extra cellular matrix (Janisiewicz, 1988; Lima *et al*., 1998; Chan and Tian, 2005), competition for nutrients and space (Janisiewicz *et al*., 2000), siderophores production and direct interaction with the pathogen (Neilands, 1981; Schwyn and Neilands, 1987; Buyer *et al*., 1989), and/or volatile production (Fravel, 1988)] are involved. Mode of actions of some microbial antagonists are depicted in appendix 1, table 4. Although several modes of action have been described for biopesticides, all mechanisms have not been fully elucidated (El-Ghaouth *et al*., 2002). It is therefore essential to elucidate the mode of action of each and single new biopesticide. Competition for nutrients, space and induction of host resistance are mechanisms demonstrated by many researchers (Janisiewicz and Korsten, 2000, 2002; Porat *et al*., 2002; Plaza *et al*., 2004) and are currently used as a major criterion for selection of new biocontrol agents for postharvest applications.

An important consideration in pre- and postharvest application of biocontrol agents is the ability of the microorganism to survive at sufficient population levels on fruit surfaces after application and rapid colonization of wound sites by organisms competing with the pathogen for nutrients and/or space (Janisiewicz *et al*., 2000). In order to be a successful competitor at the wound site and colonize the area, the antagonist must have the ability to adapt more effectively than the pathogen to various environmental conditions such as low concentrations of nutrients, varying range of temperatures and pH (Janisiewicz *et al*., 2000; Nunes *et al*., 2001). During the last decade research on citrus biocontrol focused on microorganisms colonizing the wound site and competing with pathogens for nutrients. Among these are

Cryptococcus infirmo-miniatus, *Rhodotorula glutinis* (Chand-Goyl and Spotts, 1996), *Cryptococcus laurentii* (Roberts, 1990) and *Candida oleophila* (Hofstein *et al*., 1994) all effective against *Penicillium expansum* and *Botrytis cinerea* (causal agents of blue mould and gray moulds, respectively). *Debaryomces hansenii* (Chalutz and Wilson, 1990) has also been developed against green and blue moulds as well as sour rot.

On the other hand, the induction of host resistance is one of the mechanisms involved via the activation of the key regulatory enzyme, phenylalanine ammonia lyase (PAL) and/or peroxidase (PO) towards the synthesis of soluble and/or insoluble phenolics, respectively (Harborne, 1964; Porat *et al*., 2002; Poppe *et al*., 2003). Citrus peel produced a secondary metabolite, citral, which is believed to influence fruit resistance to disease attack (Rodov *et al*., 1995). Application of antagonists and/or natural plant products on citrus fruits could involve a series of reaction steps, which could alter the amount and activity of citral.

Therefore, understanding the mode(s) of action of effective biocontrol agents is important both for improving their performance through the development of formulations enhancing the expression of useful traits, and to establish screening criteria for searching for new potential antagonists. The general outline for antagonist development and registration for use is described in figure 2.5.

2.3.2.2.3 Plant extracts as a biological control

The use of plant extracts has long been identified as a traditional means to control plant diseases (Ark and Thompson, 1959; Cowan, 1999). However, the actual use of these products in plant disease control has only recently become an important field of study (Obagwu, 2003). The family of higher plants and shrubs, particularly of tropical flora has been shown to provide potential source of naturally produced inhibitory chemicals (Kubo and Nakanishi, 1979). The natural products of plant extracts such as volatile chemicals (Wilson *et al*., 1987; Dixit *et al*., 1995; Poswal, 1996; Dudareva *et al*., 2004), essential oils (Reuveni *et al*., 1984; Tiwari *et al*., 1988; Poswal, 1996; Meepagala *et al*., 2002; Singh *et al*., 2004) and phenolic compounds (Harborne, 1964; Regnier and Macheix, 1996; Tripathi *et al*., 2002) has been used successfully to control postharvest diseases of some agricultural crops, stored fruits, vegetables and food commodities. Moreover, the anti-fungal properties of garlic (*Allium sativum* L) have also been reported (Bisht and Kamal, 1994; Obagwu *et al*. 1997; Sinha and Saxena, 1999; Obagwu, 2003) to control fungal infestations.

Mammed (2002) has reported the strong anti-fungal activity of a non-identified plant species "muka ajua" of Ethiopia, which has been used in grain storage. Further studies made on the natural products of Ethiopian medicinal plants (Dagne and Abate, 1995) indicated the potential use of tropical flora as a useful source for selecting natural plant products.

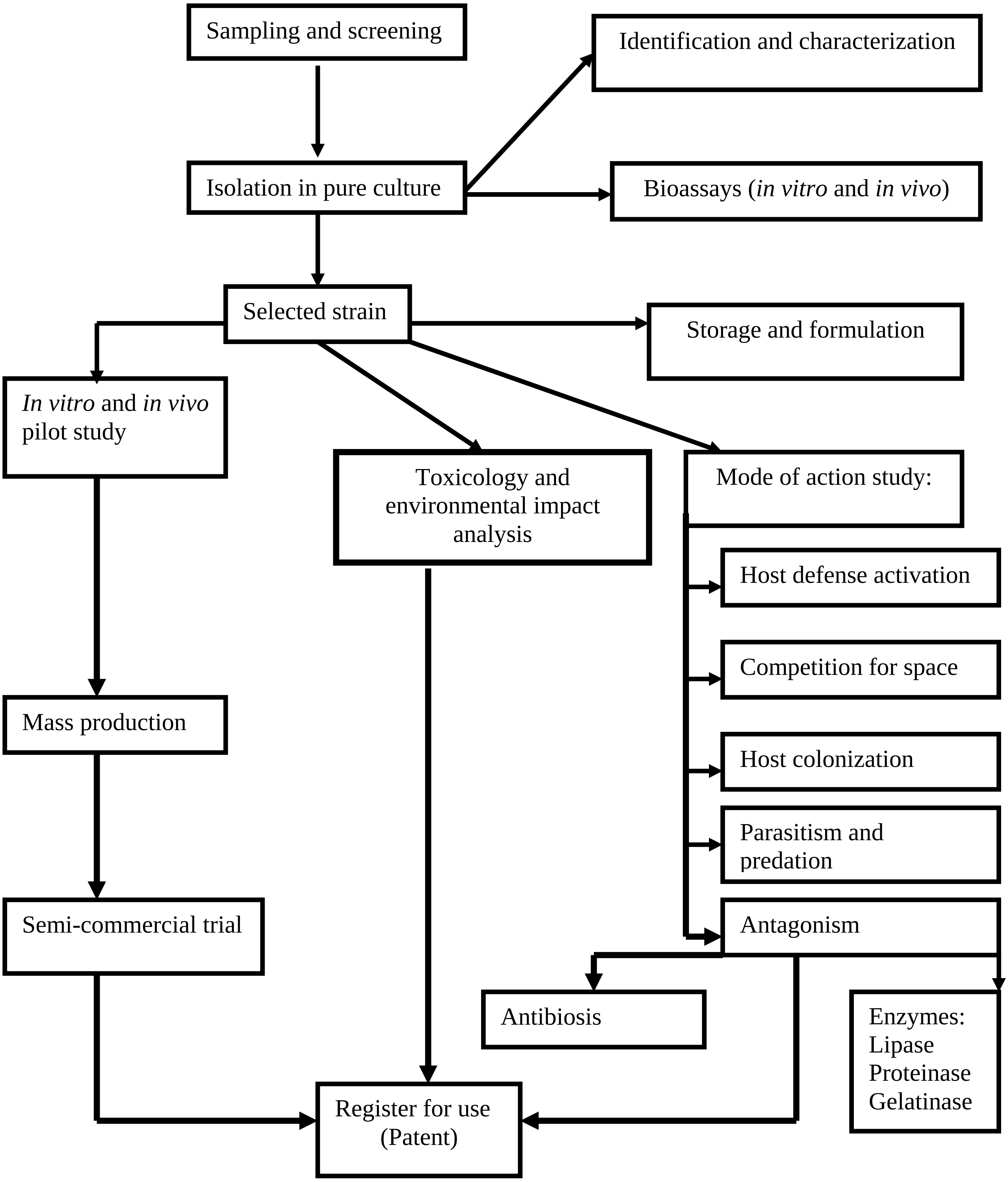

Fig. 2.5. A general outline in selection, screening and development of microbial pesticides.

The anti-helimentic activity of *Hagenia abyssinica* (Bruce) Gmel. (*Rosaceae*), anti-tumour and anti-malarial activity of *Brucea antidysenterica* Mill. (*Simaroubaceae*) (Kupchan *et al*., 1973; Phillipson and Wright, 1991), anti-leukaemic activity of *Maytenus ovatus* Loes. (*Celastraceae*) (Kupchan *et al*., 1973), analgesic and antipyretic activity of *Teclea nobilis* Delile (*Rutaceae*) and *Taverniera abyssinica* A. Rich. (*Leguminosae*) (Mascolo *et al*., 1988), antimicrobial activity of *Premna schimperi* Engl. (*Verbenaceae*) against *Staphylococcus aureus* (Habtemariam *et al*., 1993) and mulluscicidal activity of *Phytolacca dodecandra* (Lemma, 1965) are some of the many reported activities among Ethiopian flora.

Numerous antimicrobial and antifungal compounds exist naturally in plants. Plant phenolics are a diverse and abundant group of naturally occurring plant substances produced by wide range of plants (Cowan, 1999). They are characterized by the possession of aromatic rings that bear hydroxyl constituent including their functional derivatives. Most phenolic compounds are derived biosynthetically from 5-dehyderoquinate via the shikimic acid pathway or from acetate via polyketide metabolism (Fig. 2.7) (Harborne, 1964). Woody plants can synthesize and accumulate in their cells a great variety of secondary metabolites including low molecular weight phenolics (hydroxybenzoic and hydroxycinnamic acids, acetophenones, flavonoids, stilbenes and lignans (and oligo- and polymeric forms (hydrolysable and condensed cell-bound tannins and lignins) (Fig.2.6) (Harborne, 1964). The most abundant phenolics with high biomass are derived from phenylpropanoid and flavonoid biosynthesis pathways (Harborne, 1964; Robinson, 1980).

The biological significance of phenolic compounds can largely be attributed to their chemical property and reactivity (Cutler and Hill, 1994). They are generally present in the cell as glycosides or esters and are thus fairly polar (Harborne, 1964). They provide pigmentation, protection, structural support to cell wall and act as regulators of growth and development (Harborne, 1964; Robinson, 1980; Larson, 1988). Phenolic compounds make land plants adapt to UV light and ozone toxicity (Larson, 1988). Other inhibitory, stimulating and/or synergistic effects of phenolic compounds on biochemical or physiological processes and phototropism reactions mediated by phenolic photoreceptors (Towers and Abeysekera, 1984) have also been reported. Many phenolic compounds inhibit enzyme activities in a specific or non-specific manner, notably oxidative phosphorylation, ATPases and membrane transport processes (McClure, 1979). Plant phenolics play an important role as protective agents against animals and pathogens (Swain, 1977; Harborne, 1985). Toxic and inhibitory effects of phenolic compounds on cellular processes have also been observed against animals and pathogens.

Postharvest application of plant extracts on fruits has been reported to induce host resistance by altering the metabolic pathways to synthesize more phenolics in the system (Porat *et al*., 2002; Poppe *et al*., 2003; Porat *et al*., 2003). The phenolic compounds accumulated in the peel tissue have high biological activity because of their tendency towards spontaneous or enzymatic oxidation (McClure, 1979). Many phenolics exhibit toxic or inhibitory properties after oxidation to the reactive quinine form (Baranov, 1979).

2.4 Integrated control options and strategies

Biological control alone is often less effective compared with commercial fungicides or provide inconsistent control (Janisiewicz *et al*., 1992; El-Ghaouth *et al*., 2002; Leverentz *et al*., 2003). Therefore, to achieve a similar level of efficacy provided by conventional chemicals, the use of microbial antagonists integrated with commercial chemicals (Korsten, 1993; Droby *et al*., 1998), hot water (Korsten *et al*., 1991; Pusey, 1994; Auret, 2000; Nunes *et al*., 2002; Palou *et al*., 2002; Obagwu and Korsten, 2003), chloride salts (McLaughlin *et al*., 1990; Wisniewski *et al*., 1995), carbonate salts (Smilanick *et al*., 1999; El-Ghaouth *et al*., 2000; Palou *et al*., 2001; Palou *et al*., 2002; Obagwu and Korsten, 2003) and/or with natural plant extracts (Vaugh *et al*., 1993; Mattheis and Roberts, 1993; Wilson *et al*., 1997 and Obagwu *et al*., 997; Obagwu, 2003), other physical treatments such as curing and heat treatments (Leverentz *et al*., 2000; Ikediala *et al*., 2002; Plaza *et al*., 2003) provide a potential effective alternative treatments.

2.5 Postharvest disease control in Ethiopia

In Ethiopia, except for indigenous practices conducted by local people such as in North Wollo (Tisabalima, Wurgessa and Woldya), plant disease control practices entirely depend on the use of chemical pesticides applied during disease outbreaks. Relatively, high volume of chemical pesticides is utilized by Government owned citrus farms for which the annual expense for fertilizer and pesticides is estimated to be of 35% of the gross income (Appendix 1 table 5).

The use of biopesticides applied pre-and postharvestly to control fruit disease is a new technology not currently in commercial use. Therefore, the outcomes of this study will provide a base line of information for scientists in the country.

2.6 CONCLUSION

Like many other fresh fruits and vegetables, citrus are susceptible to a number of decay causing organisms. Chemical pesticides have traditionally been used to control diseases. The major problem being loss of their efficacy, alternative control options with biopesticides that showed good control have to be selected for postharvest application. The tropical flora and fauna is highly diverse and potentially useful for the search of biocontrol agents. Thus, the future can be upheld with this strategy to control pre- and postharvest diseases of crops in general, and citrus fruits in particular.

REFERENCES

Ann, P.J., Ko, W.H., Su, H.J., 2004. Interaction between Likubin bacterium and *Phytophthora parasitica* in citrus hosts. Eur. J. Plant Pathol. 110, 1-6.

Ark, P.A.,Thompson, J.P., 1959. Control of plant pathogens. The American Phytopathological Society, St. Paul MN, USA.

Arras G., DeCicoo, B., Arru, S., Lima, G., 1998. Biocontrol by yeasts of blue mould of citrus fruits and the mode of action of an isolate of *Pichia guilliermondii*. J. Hort. Sci. Biotechnol. 73, 413-418.

Auret, E.A., 2000. Control strategies for citrus postharvest diseases. Msc thesis, University of Pretoria, Pretoria, South Africa.

Babadoost, M, 2006. Oospores, sporangia and zoospores of *Phytophthora* spp. Website, http://www.apsent.org/…/phytophthora/text/fig14. htm. Accessed on January 10, 2006.

Baranov, V.I., 1979. Biological activity of oxidized phenolic compounds and their role in breakdown of IAA. Sov. Plant Physiol. 551-557.

Batta, Y.A., 2004. Postharvest biological control of apple Gray mould by *Trichoderma harzianum* Rifai formulated in an inverted emulsion. Crop Prot. 23, 19-26.

Baudoin, A.B., Eckert, J.W., 1982. Factors influencing the susceptibility of lemon to infection by *Geotrichum candidum*. Phytopathology 72, 1592-1597.

Bisht, S.S., Kamal, B.B., 1994. Garlic extract - an effective anti-fungal treatment for the control of storage rot of apple. Proc. Natl. Acad. Sci. India 64, 233-234.

Brown, G.E., 1994. Diplodia Stem-end Rot. University of Florida Cooperative Extension, USA.

Brown, G.T., 1973. Development of green mould in degreened oranges. Phytopathology 63, 1104-1107.

Browning, H.W., McGovern, R.J., Jackson, L.K., Calvert, D.V., Wardowski, W.F., 1995. Florida citrus diagnostic guide. Florida Science Source, Inc., Florida, USA, pp. 162–244.

Bull, C.T., Stack, J.P., Smilanick, J.L., 1997. *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wounds on citrus and control green and blue moulds of citrus. Biol. Cont. 8, 81-88.

Bus, V.G., Bongers, A.J., Risse, L.A., 1991. Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole and imazalil on citrus fruit from different geographic origins. Plant Dis. 75(11), 1098-1100.

Butler, E.E., Eckert, J.W., 1962. Taxonomy and physiological properties of fungus causing sour rot of citrus fruit. Phytopathology 52, 1-5.

Buyer, J., Sikora, L.J., Chaney, R.L., 1989. A new growth medium for the study of siderophore-mediated interactions. Biol. Fertil. Soils 8, 97-101.

Cappellini, R.A., Ceponis, M.J., 1984. Postharvest losses in fresh fruits and vegetables. In: Moline, H.E. (Ed.), Postharvest pathology of fruits and vegetables: Postharvest losses in perishable crops. UC, Berekeley, Agriculture Experimental Station, pp. 24-30.

Carlos, R., 1982. Manual and atlas of the Penicillia. Elsevier Biomedical Press, Netherlands.

Central Agricultural Census Commission of Ethiopia (CACCE), 2003. Country level statistical report on socio-economic characteristic of the population in agricultural households, land use, and area and production of crops (Vol. I) Central Statistical Authority, Addis Ababa, Ethiopia.

Chalutz, E., Wilson, C.L., 1990. Postharvest biocontrol of green and Blue mould and Sour rot of citrus fruit by *Debaryomyces hansenii*. Plant Dis. 74, 134-137.

Chan, Z., Tian, S., 2005. Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. Postharvest Biol. Technol. 36, 215-223.

Chand-Goyal, T., Spotts, R.A., 1996. Control of postharvest pear diseases using natural saprophytic yeast colonists and their combination with a low dosage of thiabendazole. Postharvest Biol. Technol. 7, 51-64.

Claypool, L.L., 1983. Biological and cultural aspects of production and marketing of fruits. In: O'brien, M., Cargell, B., Fridley R.B. (Eds.), Principles and practices for harvesting and handling fruits and nuts. Avi publishing company, Inc., USA. pp 15-45.

Conway, W.S., Janisiewicz, W.J., Klein. J.D., Sams, C.E., 1999. Strategy for combining heat treatment, calcium infiltration, and biological control to reduce postharvest decay of gala apple. HortScience 34, 700-704.

Coursey, D.G., Booth, R.H., 1972. The postharvest phytopathology of perishable tropical produce. Rev. Plant Pathol. 751-765.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4), 564-582.

Cutler, H.G., Hill, R.A., 1994. Natural fungicides and their delivery systems as alternatives to synthetics. In: Wilson, C.L., Wisniewski, M.E. (Eds.), Biological control of postharvest diseases. CRC Press, Roca Raton.

Dagne, A., Abate, D., 1995. Bioactive compounds from plants and higher fungi of Ethiopia. In: Hostettmann, K., Marston, A., Maillard, M., Hamburger, M. (Eds.), Phytochemistry of plants used in traditional medicine Clarendon Press, Oxford, pp. 295-312.

Davies, F.S., Albrigo, L.G., 1994. Citrus. CAB International, Great Britain.

Demoz, B.T., Korsten, L., 2006. *Bacillus subtilis* attachment, colonization, and survival on avocado flowers and its mode of action on stem-end rot pathogens. Biol. Contr. 37, 68-74.

Dixit, S.N., Chandra, H., Tiwari, R., Dixit, V., 1995. Development of a botanical fungicide against blue mould of mandarins. J. Stored Prod. Res. 31(2), 165-172.

Dixon, G.R., 1984. Control: cultural and biological methods. In: Plant pathogens and their control in horticulture. Science Horticulture Series. Macmillan press, UK. pp. 202-233.

Droby, S., Chalutz, E., Wilson, C.L., Wisniewski, M., 1989. Characterization of the biological control activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruits. Can. J. Microbiol. 35, 794-800.

Droby, S., Cohen, A., Weiss, B., Horev, B., Chalutz, E., Katz, H., Keren-Tzur, Shachnai, S., 1998. Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. Biol. Contr. 12, 97-100.

Droby, S., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E.E., Porat, R., 2002. Induction of resistance to *Penicillium digitatum* in grape fruit by the yeast biocontrol agent *Candida oleophila*. The Am. Phytopathol. Soc. 92 (4), 393-399.

Dudareva, N., Pichersky, E., Gershenzon, J., 2004. Biochemistry of plant volatiles. Plant Physiol. 135, 1893-1902.

Eckert, J.W., Ogawa, J.M., 1985. The chemical control of postharvest diseases: subtropical and tropical fruits. Ann. Rev. Phytopathol. 23, 421-454.

Eckert, J.W., Sievert, J.R., Ratnayake, M., 1994. Reduction of imazalil effectiveness against citrus green mould in California packinghouses by resistant biotypes of *Penicillium digitatum*. Plant Dis. 78, 971-974.

EI-Ghaouth, A., Smilanick, J., Brown, E., Ippoloto, A., Wilson, C.L., 2000. Application of *Candida saitoana* and glycolchitosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. Plant Dis. 84, 243-248.

El-Ghaouth, A., Smilanick, J.L., Wilson, C.L. 2000. Enhancement of the performance of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest of decay of apple and citrus fruit. Postharvest Biol.Technol. 19, 103-110.

EI-Ghaouth, A., Wilson, C.L, Wisniewski, M., Droby, S., Smilanick, J.L., Korsten, L., 2002. Biological control of postharvest disease of citrus fruits. In: Gnanamanickam, S.S. (Ed.), Biological control of crop diseases, Mared Dekker, Inc., New York, pp. 289-312.

Eyob, S., 1997. Rehabilitation of old citrus orchards at Werer (Abstract). Rev. Plant Pathol. 76(7), 741.

Food and Agricultural Organization, 1965. Production year book. Rome, Italy 19, 117.

Food and Agricultural Organization, 2004. “FAOSTAT Agricultural Data” of World citrus area harvest and production statistics. http://apps.fao.org/page/collections?subset=agriculture. Accessed on September 24 2004.

Fravel, D.R., 1988. Role of antibiosis in the control of plant diseases. Ann. Rev. Phytopathol. 26, 75-91.

Futch, S.H., Timmer, L.W., 2001. A guide to citrus disease identification. University of Florida, IFAS Extension.

Gmitter, F.G., Jr, Hu, X., 1990. The possible of Yunnan, China in the origin of contemporary citrus species (*Rutaceae*). Econ. Bot. 44, 267-277.

Gorinstein, S., Martin-Belloso, O., Park, Y., Haruenkit, R., Lojek, A., Milan, I., Caspi, A., Libman, I., Trakhtenberg, S., 2001. Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 74 (3), 309-315.

Govender, V., Korsten, L., 2006. Evaluation of different formulations of *Bacillus licheniformis* in mango packhouse trials. Biol. Contr. 37, 237-242.

Habtemariam, S., Gary, A.I., Waterman, P.G., 1993. A new antibacterial Sesquiterpene from *Premna oligotricha*. J. Nat. Prod. 56, 140-143.

Harborne, J.B., 1964. Biochemistry of phenolic compounds. Academic Press, London. pp. 295-361.

Harborne, J.B., 1985. Phenolics and plant defence. In: Van-Sumere, C.F., Lea, P.J. (Eds.). Annual Proceedings of Phytochemical Society of Europe 25 Clarendon Press, Oxford, pp. 393-408.

Hofstein, R., Fridlender, B., Chalutz, E., Droby, S., 1994. Large scale production and pilot testing of biological control agents for postharvest diseases. In: Wilson, C.L., Wisniewski, M.E. (Eds.), Biological control of postharvest diseases-Theory and practice. CRC Press, Boca Raton, FL, pp. 89-100.

Howard, F.S., 1936. Citrus diseases and their control. McGraw-Hill Co. Inc., New York.

Huang, Y., Wild, B.L., Morris, S.C., 1992. Postharvest biological control of *Penicillium digitatum* decay on citrus fruit by *Bacillus pumilus*. Ann. App. Biol. 120, 367-372.

Ikediala, J.N., Hansen, J.D., Tang, J., Drake, S.R., Wang, S., 2002. Development of a saline water immersion technique with RF energy as a postharvest treatment against codling moth in cherries. Postharvest Biol.Technol. 24, 209-221.

Ismail, M., Zhang, J., 2004. Postharvest citrus diseases and their control. Outlooks Pest Manag. 1(10), 29-35.

Janisiewicz, W.J., 1985. Biological control of postharvest disease of pome fruits. Phytopathology 77, 481-485.

Janisiewicz, W.J., 1987. Postharvest biological control of blue mould on apple. Phytopathology 77, 481-185.

Janisiewicz, W.J., 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. Phytopathology 78, 194-198.

Janisiewicz, W.J., 1994. Enhancement of Biocontrol of Blue mould with the natural analog 2 - Deoxy D - Glucose on apples and pears. Appl. Environ. Microbiol. 60 (8), 2671-2676.

Janisiewicz, W.J., Korsten, L., 2000. Control of postharvest diseases and microbial spoilage of vegetables by application of microbes. In: Bartz, J. (Ed.), The physiology and microbiology of vegetables after harvest. Chapter 23, Marel Dekker, New York.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Ann. Rev. Phytopathol. 40, 411-44.

Janisiewicz, W.J., Tworkoski, T.J., Sharer, C., 2000. Characterizing the mechanism of biological control of postharvest diseases on fruit with a simple method to study competition for nutrients. Phytopathology 90, 1196–1200.

Janisiewicz, W.J., Usall, J., Bors, B., 1992. Nutritional enhancement of biological control of blue mould on apples. Phytopathology 82, 1364 -1370.

Koomen, I., Jeffries, P., 1993. Effects of antagonistic microorganisms on the postharvest development of *Colletotrichum gloeosporioides* on mango. Plant Pathol. 42, 230-237.

Korsten, L. 1993. *Biological control of avocado fruit diseases*. PhD Thesis, University of Pretoria, Pretoria, South Africa.

Korsten, L., De-Jager, E.S., 1995. Mode of action of *Bacillus subtilis* for control of avocado posthavest pathogens. South African Avocado Growers' Association Year Book 18, 124-130.

Korsten, L., De-Jager, E.E., Paul, I., Obagwu, J., El-Ghaouth, A., 2000. Altenative control of citrus Postharvest disease. Poster presented at the International Society of citriculture at Orlando, Florida, USA, 3-7, December 2000.

Korsten, L., De-Villers, E.E., Wehner, F.C., Koteze, J.M., 1997. Field sprays of *Bacillus subtilis* and fungicides for control of postharvest fruit diseases of avocado in South Africa. Plant dis. 81, 455-459.

Korsten, L., Van Harmelen, M. W. S., Heitmann, A., De Villers, E.E., De-Jager, E.S. 1991. Biological control of postharvest mango diseases. South African Mango Growers Association Yearbook 11, 65-67.

Kotze, J.M., 1993. Black spot: citrus fungal disease. In: Whiteside, J.O., Garnsey, S.M., Timmer, L.W. (Eds.), Compendium of citrus diseases. APS press, USA.

Kubo, I., Nakanishi, K., 1979. Some terpenoid insect antifeedants from tropical plants. In: Geissbuhaler, H., Pergamon (Eds.), Press, Oxford. pp. 284-294.

Kupchan, S.M., Britton, R.W., Ziegler, MF., Sigel, C.W., 1973. Bruceantin, a new potent anti-leukaemic simaroubolide from *Brucea antidysenterica*. J. Organic Chem. 38, 178-179.

Kuramoto, T., 1979. Survival of *Penicillium digitatum* and *P. italicum* in soil. Bull. Fruit Tree Res.Stn., Ser. B. Okitsu (Jap.) 6, 137-149.

Larson, R.A., 1988. The antioxidants of higher plants. Phytochemistry 27, 969-978.

Lemma, A., 1965. A preliminary report on the molluscicidal property of “Endod” (*Phytolacca dodecandra*). Eth. Med. J. 3, 187-90.

Leverentz, B., Janisiewicz, W.J., Conway, W.S., Saftner, R.A., Fuchs, Y., Sams, E.E., Camp, M.J., 2000. Combining yeasts or a bacterial biocontrol agent and heat treatment to reduce postharvest decay of ‘Gala’ apples. Postharvest Biol.Technol. 21, 87-94.

Leverentz, B., Conway, W.S., Janisiewicz, W.J., Saftner, R.A., Camp, M.J., 2003. Effect of combining MCP treatment, heat treatment, and biocontrol on the reduction of postharvest decay of ‘Golden Delicious’ apples. Postharvest Biol.Technol. 27, 221-233.

Lima, G., De Curtis, F., Castoria, R., De Cicco, V. 1998. Activity of the yeasts *Cryptococcus laurentii* and *Rhodotorula glutinis* against postharvest rots on different fruits. Biocontrol Sci. Technol. 8, 257-267.

Lipsky, G.A., 1962. Ethiopia: its people, its society, its culture. HRAF Press, New Haven, pp. 376.

Mammed, A.A., 2002. Mycoflora and mycotoxins of major cereal grains and antifungal effects of selected medicinal plants from Ethiopia. PhD thesis, Gottingen University, Germany.

Mascolo, N., Pinto, A., Capasso, F., Yenesew, A., Dagne, E., 1988. Antipyretic and analgesic studies of the ethanolic extracts of *Teclea nobilis* Delile. Phytother. Res. 2, 154-156.

Mattheis, J., Roberts, R., 1993. Fumigation of sweet cherry (*Prunus avium* ‘Bing’) fruit with low molecular weight aldehydes for postharvest decay control. Plant Dis. 77, 810-814.

McClure, J.M., 1979. Physiology of phenolic compounds. In: Swain, T., Harborne, J. B., Van-Sumere C.F. (Eds.), Plenum Press, New York, pp. 525-556.

Mc-Laughlin, R.J., Wisniewski, M.E., Wilson, C.L. Chalutz, E., 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. Phytopathology 80, 456-461.

Meepagala, K.M., Sturtz, G., Wedge, D.E., 2002. Antifungal constituents of the essential oil fraction of *Artemisia drancunculus* L. var. dracunculus. J. Agric. Food Chem. 50, 6989-6992.

Mercier, J., Wilson, C.L., 1994. Colonization of apple wounds by naturally occurring microflora and introduced *Candida oleofila* and their effect on infection by *Botrytis cinerea* during storage. Biol. Contr. 4, 138-144.

Montesinos, E., 2003. Development, registration and commercialisation of microbial pesticides for plant protection. Intro. Microbiol. 6, 245-252

Morgan, M., 2006. Moulds. http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artjan99/mmould.html
Accessed on April 23 2006.

Neilands, J.B., 1981. Microbial iron compounds. Ann. Rev. Biochem. 50, 715-731.

Norman, C., 1988. EPA sets new policy on pesticide cancer risks. Science 242, 366-367.

Nunes, C., Usall, J., Teixido, N., Abadias, M., Immaculada, V., 2002. Improved control of postharvest decay of pears by the combination of *Candida sake* (CPA- 1) and Ammonium Molybdate. Phytopathology 3 (92), 281-287.

Nunes, C., Usall, J., Teixido, N., Miro, M., Vinas, I., 2001. Nutritional enhancement of biocontrol activity of *Candida sake* (CPA-1) against *Penicillium expansum* on apples and pears. Eur. J. Plant Pathol. 107, 543-551.

Obagwu, J., 2003. Developing biopesticides for control of citrus fruit pathogens of importance in global trade. PhD thesis, University of Pretoria, Pretoria, South Africa.

Obagwu, J., Emechebe, A. M., Adeoti, A.A., 1997. Effect of extracts of garlic (*Allium sativum* L.) bulb and neem (*Azadirachta indica* Juss) seed on the mycelial growth and sporulation of *Colletotrichum capsici*. J. Agri. Technol. 5, 51-55.

Obagwu, J., Korsten, L., 2003. Integrated control of citrus green and blue moulds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water. Postharvest Biol. Technol. 28, 187-194.

Oberholzer, P.C.J., 1969. Citrus culture in Africa south of the Sahara. Proc. Int. Citrus Symp. 1, 111-119.

Palou, L., Smilanick, J.L., Usall, J., Virias, I., 2001. Control of postharvest blue and green moulds of oranges by hot water, sodium carbonate and sodium bicarbonate. Plant Dis. 85, 371-376.

Palou, L., Usall, J., Munoz, J.A., Smilanick, L., Vinas, I., 2002. Hot water, sodium carbonate, and sodium bicarbonate for the control of postharvest green and blue molds of clementine mandarins. Postharvest Biol.Technol. 24, 93-96.

Pang, X., Qun Zhang, Z.Q., Huang Xue, M., 2002. Bilogical control of postharvest diseases of fruits and vegetables. J. Trop. Subtrop. Bot. 10, 186–192.

Phillipson, J.D, Wright, W., 1991. Can ethnopharmacology contribute to the development of anti-malarial agents? J. Ethnopharmacol. 32, 155-165.

Plaza, P., Usall, J., Teixido, N., Vinas, I., 2004. Effect of water activity and temperature on competing abilities of common postharvest citrus fungi. Int. J. Food Microbiol. 90, 75-82.

Plaza, P., Usall, J., Torres, R., Lamarca, N., Asensio, A., Vinas, I., 2003. Control of green and blue mould by curing on oranges during ambient and cold storage. Postharvest Biol. Technol. 28, 195-198.

Poppe, L., Vanhoutte, S., Hofte, M., 2003. Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. Eu. J. Plant Pathol. 109, 963-973.

Porat, R., McCollum, T.G., Vinokur, V., Droby. S., 2002. Effects of various elicitors on the transcription of a β-1, 3- endoglucanase gene in citrus fruit. J. Phytopathol. 150, 70-75.

Porat, R., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E.E., Droby, S., 2003. Induction of resistance to *Penicillium digitatum* in grape fruit by β-aminobutyric acid. Eu. J. Plant Pathol. 109: 901-907.

Poswal, M.A.T., 1996. Antifungal activity of natural plant oils against *Alternaria citri*, the navel-end rot pathogen. Proc. Int. Soc. Citri. 478-481.

Pusey, P.L., 1994. Enhancement of bocontrol agents for postharvest diseases and their integration with other control strategies. In: Wilson, E.L.,Wisniewski, M.E. (Eds.), Biological control of postharvest diseases CRC press, Bock Raton, pp. 77-88.

Pusey, P.L., Wilson, C.L., 1984. Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. Plant Dis. 68(9), 753-756.

Regnier, T., Macheix, J.J., 1996. Changes in wall-bound phenolic acids, phenylalanine and tyrosine ammonia-lyases, and peroxidases in developing durum wheat grains (*Triticum turgidum* L. var. Durum). J. Agri. Food Chem. 44, 1727-1730.

Reimann, S., Deising, H.B., 2000. Fungicides: risk of resistance development and search for new targets. Arch. Phytopathol. Plant Prot. 33, 329-349.

Reuveni, R., Fleischer, A., Putievski, E., 1984. Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes. Phytopathology 10, 20-22.

Roberts, R.G., 1990. Postharvest biological control of Gray mould of apple by *Cryptococcus laurentii*. Phytopathology 80, 526-530.

Robinson, T., 1980. The organic constituents of higher plants, 4. Cordus Press, Massachusetts, USA, pp. 54-86.

Rodov, V., Ben-Yehoshua, S., Fang, D.Q., Kim, J.J., 1995. Preformed antifungal compounds on Irmon fruit; citral and its relation to disease resistance. J. Agric. Food Chem. 43, 1057-1061.

Salunkhe, D.K., Desai, B.B., 1984. Postharvest biotechnology of fruits (vol. I). CRC Press, Florida.

Samson, J.A., 1980. *Tropical fruits* - Tropical agricultural series. Longman Inc., New York, pp. 64-118.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47-56.

Scora, R.W. 1975. The history and origin of citrus. Bull.Torrey Bot. Club 102, 369-375.

Seifu, G.M., 2003. Status of commercial fruit production in Ethiopia. Ethiopian Agricultural Research organization (EARO), Addis Abba, Ethiopia.

Shachnal, A., Chalutz, E., Droby, S., Cohen, L., Weiss, B., Daus, A.Q., Katz, H., Bercovitz, A., Keren-Tzur, M., Binyamini, Y., 1996. Commercial use of Aspire^{TM} for the control of postharvest decay of citrus fruit in the packinghouses. Proc. Int. Soc. Citri. 2, 1174-1177.

Singh, G., Marimuthu, P., Catalan, C., de-Lampasona, M.P., 2004. Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract. J. Sci. Food Agric. 84, 1878-1884.

Sinha, P., Saxena, S.K., 1999. Inhibition of fruit rot fungus and fruit fly by leaf extracts of onion and garlic. Indian J. Agric. Sci. 69, 651-653.

Smilanick, J.L., Denis-Arrue, R., 1992. Control of green mould of lemons with *Pseudomonas* species. Plant Dis. 76, 481-485.

Smilanick, J.L., Margosan, D.A., Mlikota, F., Usall, J., Michael, I.F., 1999. Control of citrus green mould by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis. 83, 139-145.

Sommer, N.F., Ewards, D., 1992. Postharvest disease of selected commodities 15, 117-166.

Spadaro, D., Vola, R. Piano, S. Gullino, M.L., 2002. Mechanisms of action and efficacy of four isolates of the yeast *Metschnikowia pulcherrima* active against postharvest pathogens on apples. Postharvest Biol.Technol. 24, 123-134.

Swain, T., 1977. Secondary compounds as protective agents. Ann. Rev. Plant Physiol. 28, 479-501.

Swingle, W.T., 1943. The botany of citrus and its wild relatives of the orange subfamily (family *Rutaceae*, subfamily *Aurantiodeae*). In: Webber, H.J., Batchelor, L.D. (Eds.), The citrus industry, vol. 1. University of California Press, Berkeley, pp. 129-474.

Timmer, L.W., Duncan, LW., 1999. Citrus health management, American Phytopathological Society Press, St. Paul, MN.

Tiwari, R., Mishra, D.N., Upadhyay, P.S., 1988. Efficacy of some plant volatiles for the control of black mould of onion caused by *Aspergillus niger* Van Tiegh during storage. Natl. Acad. Sci. Lett. 11, 345-347.

Timmer, L.W., Menge, J.A., 1988. *Phytophthora*-induced diseases. In: Whiteside, J.O., Garnsey, S.M., Timmer, L.W. (Eds.), Compendium of citrus diseases APS Press, St. Paul, Minnesota, USA, pp. 22-24.

Towers, G.H.N., Abeysekera, B., 1984. Cell wall hydroxycinnamate esters as UV-A receptors in phototropic responses of higher plants- A new hypothesis. Phytochemistry 23, 951-952.

Tripathi, P., Dubey, N.K., 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol. Technol. 32, 235-245.

Tripathi, P., Dubey, N.K., Pandey, V.B., 2002. Kaempferol: the antifungal principle of *Acacia nilotica* Linn. Del. Indian Bot. Soc. 81, 51-54.

Vaugh, S.F., Spencer, G.F., Shasha, S., 1993. Volatile compounds from raspberry and straw berry fruit inhibit postharvest decay fungi. J. Food Sci. 58, 793-796.

Vero, S., Mondino, P., Burgueno, J., Soubes, M. and Wisniewski, M., 2002. Characterization of biocontrol activity of two yeast strains from Uruguay against blue mould of apple. Postharvest Biol.Technol. 26, 91–98.

Wardowski, W.F., Brown, G.E., 2001. Postharvest decay control recommendations for Florida citrus fruit. Cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida, pp. 1-7.

Webber, J.H., Reuther, W., Lawton, H.W., 1967. History and development of the citrus industry In: Davies, F.S., Albrigo, L.G. (Eds.), Crop production science in Horticulture: Citrus, Cab International, and Wallingford, UK, pp. 1-242.

Whipps, J. M., McQuilken, M.P., 1993. Aspects of biocontrol of fungal plant pathogens. In: Jones D.G. (Ed.), Exploitation of microorganisms. Chapman and Hall, London, pp. 45-79.

Whiteside, J.O., Garnsey, S.M., Timmer, L.W., 1988. Compendium of citrus diseases. American Phytopathological Society Press MN, USA.

Whiteside, J.O., Garnsey, S.M., Timmer, L.W., 1993. Compendium of citrus diseases. The American Phytopathological Society, APS press, USA.

Wills, R., McGlasson, B., Graham, D., Joyce, D., 1998. Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals. Cab International, Sydeny, Australia.

Wilson, C.L., Franklin, J.D., Pusey, P.L., 1987. Biological control of *Rhizopus* rot of peach with *Enterobacter cloacae*. Phytopathology 77, 303-305.

Wilson, C.L., Wisniewski, M. E., 1989. Biological control of postharvest diseases of fruits and vegetables: An emerging technology. Ann. Rev. Phytopathol. 27, 425- 441

Wilson, C.L., El-Ghaouth, A., Solar, J., Wisniewski, J.M., 1997. Rapid evaluation of plant extracts and essential oils for fungicidal activity against *Botrytis cinerea*. Plant Dis. 81, 204-210.

Wisniewski, M.E., Droby, S., Chalutz, E., Eilam, Y., 1995. Effect of Ca^{+2} and Mg^{+2} on *Botrytis cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of *Candida oleophila*. Plant Pathol. 44, 1016-1024.

Wisniewski, M. E., Wilson, C.L., 1992. Biological control of postharvest diseases of fruits and vegetables: recent advances. HortScience 27(2), 94-98.

Zhang, H., Zheng, X., Fu, C., Xi, Y., 2005. Postharvest biological control of Gray mould rot of pear with *Cryptococcus laurentii*. Postharvest Biol.Technol. 35, 79-86.