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REVIEW ARTICLE

Alternative methods for the control of postharvest citrus diseases

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Summary

The postharvest diseases of citrus fruit cause considerable losses during storage and transportation. These diseases are managed principally by the application of synthetic fungicides. However, the increasing concern for health hazards and environmental pollution due to chemical use has required the development of alternative strategies for the control of postharvest citrus diseases. Management of postharvest diseases using microbial antagonists, natural plant-derived products and Generally Recognized As Safe compounds has been demonstrated to be most suitable to replace the synthetic fungicides, which are either being banned or recommended for limited use. However, application of these alternatives by themselves may not always provide a commercially acceptable level of control of postharvest citrus diseases comparable to that obtained with synthetic fungicides. To provide more effective disease control, a multifaceted approach based on the combination of different postharvest treatments has been adopted. Actually, despite the distinctive features of these alternative methods, several reasons hinder the commercial use of such treatments. Consequently, research should emphasize the development of appropriate tools to effectively implement these alternative methods to commercial citrus production.

Introduction

Citrus is one of the most widely produced fruit globally. It is grown commercially in more than 137 countries around the world (Ismail and Zhang 2004). The contribution of the citrus industry to the world economy is enormous, and it provides jobs to millions of people around the world in harvesting, handling, transportation, storage and marketing operations. The importance of citrus fruit is attributed to its diversified use, which is widely consumed either as fresh fruit or as juice.

Due to their higher water content and nutrient composition, citrus fruit is very susceptible to infection by microbial pathogens during the period between harvest and consumption (Tripathi and Dubey 2003). Citrus fruits are usually quite acidic, in the pH range 2·2–4. For this reason, so the most of the decay in harvested fruits is caused by fungi. No bacterial postharvest disease of commercial importance is reported on citrus fruit. Contamination and infection by pathogenic fungi occur at different stages in the field and after harvest and usually follows mechanical injury of the fruit, which allows entry of these micro-organisms. Postharvest decays of fruit can also originate from latent infections occurring in the orchard such as black rot caused by *Alternaria alternata* pv. *citri*, brown rot caused by *Phytophthora citrophthora* and anthracnose caused by *Colletotrichum gloeosporioides.*

In developing and nondeveloped countries, high losses result from inadequate storage facilities and improper transport and handling (Ladaniya 2008). Citrus fruits are susceptible to a number of postharvest diseases that cause significant losses during the postharvest phase. Nevertheless, the most common and serious diseases that affect citrus fruit are green and blue moulds caused, respectively, by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer, followed in importance by sour rot caused by *Geotrichum citri-aurantii* Link ex Persn (Caccioni *et al.* 1998; Palou *et al.* 2002; Zheng *et al.* 2005). These pathogens are strict wound pathogens that can infect the fruit in the grove, in the packinghouse, or during subsequent handling and storage (Palou *et al.* 2008). The fungal inoculum is practically always present on the surface of fruit during the season and after harvest can build up to high levels unless appropriate packinghouse sanitization measures are adopted (Kanetis *et al.* 2007). Fruit infection by these fungi is enhanced during the fruit degreening operation and during wet and rainfall seasons (Liu *et al.* 2009b). Decay is also more prevalent as fruit increases in maturity, and at favourable temperatures and humidity (Baudoin and Eckert 1985).

Currently, synthetic fungicides are the primary means of controlling postharvest diseases of citrus fruit, especially imazalil (IZ), thiabendazole (TBZ), sodium orthophenyl phenate (SOPP), fludioxonil (FLU), pyrimethanil or different mixtures of these compounds (Ismail and Zhang 2004; Smilanick *et al.* 2005; Palou *et al.* 2008). However, the postharvest use of these fungicides is subject to registration and permission for use in various countries. Continuous use of these fungicides has resulted in the appearance of isolates of fungi with multiple fungicide resistances, which further complicate the management of the diseases (especially *Penicillium* rots) (Droby *et al.* 2002; Mercier and Smilanick 2005; Boubaker *et al.* 2009). In addition, these fungicides are not effective against all important pathogens. Indeed, sour rot is difficult to control with IMZ and TBZ (Suprapta *et al.* 1997; Mercier and Smilanick 2005). The synthetic fungicide guazatine is the only commercial product that can control sour rot (Brown 1988). However, this fungicide is no longer authorized in Morocco and several other countries. Furthermore, the use of fungicides is increasingly becoming restricted owing to stringent regulation, carcinogenicity, high and acute residual toxicity, long degradation period, environmental pollution and growing public concern about chemical residues in fruit (Tripathi and Dubey 2003; Palou *et al.* 2008).

Therefore, the challenge is to develop safer and eco-friendly alternative strategies of controlling citrus postharvest diseases, which pose less risk to human health and environment. Recently, several promising biological approaches have been proposed as potential alternatives to synthetic fungicides for the control of postharvest citrus disease. These biological control strategies include as follows: (i) use of antagonistic micro-organisms; (ii) application of naturally derived bioactive compounds; and (iii) induction of natural resistance. Among these biological approaches, the use of the microbial antagonists, either alone or as part of an integrated disease management policy, is quite promising and gaining popularity among consumers (Droby *et al.* 2002). Interestingly, most of the antagonistic micro-organisms are isolated from fruit surfaces as epiphytic microbial population. Continual laboratory experimentation followed by packinghouse experiments are needed to establish excellent biocontrol agents particularly against postharvest fungal pathogens.

The second approach for disease control is the use of natural plant-derived compounds. Indeed, these compounds have gained popularity and scientific interest for their antibacterial and antifungal activities (Tripathi and Dubey 2003; Du Plooy *et al.* 2009; Liu *et al.* 2009b; Gatto *et al.* 2011; Talibi *et al.* 2011a,b; Talibi *et al.* 2012a,b). The use of natural plant products is an interesting alternative or a complementary control method because of their antifungal activity, nonphytotoxicity, systemicity and biodegradability (Tripathi and Dubey 2003; Ameziane *et al.* 2007; Gatto *et al.* 2011).

However, commercially speaking, the application of these biological control methods may not always provide acceptable levels of control of postharvest citrus diseases. It is possible to increase the efficiency of these methods by combining them with other postharvest treatments. Indeed, different disease management strategies have been integrated to provide more effective disease control in comparison with a single approach. Low-toxicity chemicals, particularly common food additives and Generally Regarded As Safe (GRAS) compounds, have been evaluated for their efficacy for the control of citrus pathogens. Strictly speaking, GRAS compounds do not fall into the category of organic ingredients, but they are much less harmful than many other inorganic chemicals.

The purpose of this study is to provide an updated overview of the published data on alternative control methods to conventional chemical fungicides for the control of postharvest citrus pathogens (Janisiewicz and Korsten 2002; Spadaro and Gullino 2004; Palou *et al.* 2008; Droby *et al.* 2009; Sharma *et al.* 2009; Nunes 2012; Liu *et al.* 2013).

Integrated strategies to control postharvest citrus diseases

Antagonistic micro-organisms as biocontrol agents

It has been demonstrated that natural microbial antagonists exist on fruit surfaces that can suppress disease development (Wilson and Wisniewski 1989). The use of antagonistic micro-organisms for controlling the postharvest diseases of citrus fruit is based on two approaches: (i) the use of natural epiphytic antagonists that already exist on fruit surfaces, and (ii) the artificial introduction of selective microbial antagonists that control postharvest diseases. The importance of naturally occurring microbial antagonists is revealed when washed fruits develop more rot than unwashed ones (Wilson and Wisniewski 1989). Chalutz and Wilson (1990) found that washed, dried and stored citrus fruit get infected much more rapidly than unwashed fruit. This suggests that the resident epiphytic microflora on citrus fruit is capable of controlling citrus diseases. Moreover, according to their origin, these naturally occurring antagonists are more apt to gain public acceptance. Several antagonistic micro-organisms, found to be effective in controlling citrus diseases in the postharvest phase, were isolated from the surface of citrus fruit (Wilson and Chalutz 1989; Borras and Aguilar 1990; Chalutz and Wilson 1990; Droby *et al.* 1998; Taqarort *et al.* 2008) (Table 1). Currently, available microbial antagonists were developed for the control of decay originating mainly from active infection of fruit wounds and not from quiescent infections. Furthermore, until now, biological control is clearly not going to completely replace the use of fungicides for the control of the postharvest citrus diseases, but it will increasingly be a part of integrated control programmes for major fungal diseases.

Yeasts as biocontrol agents

Treatment of citrus fruit with antagonistic yeasts is one of the best alternatives to control postharvest diseases (Janisiewicz and Korsten 2002). This importance is attributed to several positive characteristics that make yeasts effective microbial agents for the control of postharvest diseases of fruit. First, yeasts can colonize the surface of fruit for long period even under dry conditions (Janisiewicz 1987). Second, yeasts produce extracellular polysaccharides, which enhance their survivability and restrict the growth of pathogen propagules. Third, yeasts can use nutrients rapidly and proliferate at a high rate (Sharma *et al.* 2009). Effective control of citrus fruit decay was observed with yeasts such as *Candida guilliermondii* (syn.:*Pichia guilliermondii*), *Pichia anomala*, *Candida saitoana*, *Candida famata*, *Candida oleophila*, *Candida sake*, *Aureobasidium pullulans* and *Kloeckera apiculata* (Wilson and Chalutz 1989; Chalutz and Wilson 1990; Lima *et al.* 1997; El-Ghaouth *et al.* 2000; Droby *et al.* 2002; Taqarort *et al.* 2008) (Table 1). Actually, two products based on antagonistic yeasts are commercially available to manage postharvest diseases: Biosave (*Pseudomonas syringae* Van Hall) and 'Shemer' (*Metschnikowia fructicola* Kurtzman & Droby) (Droby *et al.* 2009). Two early yeast-based products AspireTM (Ecogen, Philadelphia, PA) and YieldPlus (AnchorYeast, Cape Town, South Africa) are no longer available (Droby *et al.* 2009). A few others are still in different stages of commercial improvement and expected to be released in the market in the future. However, application of these biocontrol products alone did not provide commercially acceptable control of fruit diseases. The biocontrol ability of these antagonists could be enhanced by manipulation of the environment, using mixtures of beneficial organisms, physiological and genetic enhancement of the biocontrol mechanisms and integration of biocontrol with other methods such as low doses of fungicides and controlled atmosphere storage (Spotts *et al.* 2002).

Bacteria as biocontrol agents

Use of bacteria for postharvest disease control of citrus has focused on their application as biofungicides. Plant-associated bacteria are ubiquitous in most plant species and can be isolated from surface plant tissues, soils, roots and the rhizosphere of various plants. Moreover, endophytic bacteria are of interest as biocontrol agents because of their rhizosphere competence and their ability to colonize internal tissues of plants and thus provide an internal defence against pathogens (Liu *et al.* 2009a). Antagonistic bacteria are well known for their production of substances with antifungal and antibacterial properties (Smilanick and Denis-Arrue 1992; Leelasuphakul *et al.* 2008; Lucon *et al.* 2010; Yánez-Mendizábal *et al.* 2011). Significant advances in the control of postharvest citrus diseases have been achieved by the use of bacterial antagonists such as *Ps. syringae*, *Ps. fluorescens*, *Burkolderia* (*Pseudomonas*) *cepacia*, *Bacillus subtilis*, *B. thuringiensis*, *Pantoea agglomerans, Enterobacter cloacae* and *Serratia plymuthica* (Singh and Deverall 1984; Smilanick and Denis-Arrue 1992; Bull *et al.* 1998; Meziane *et al.* 2006; Cañamás *et al.* 2008; Lucon *et al.* 2010) (Table 1). However, only one species of bacteria has been mass-produced and commercialized under the trade name Bio-Save 100 and 110 based on a strain of *Ps. syringae,* to control postharvest citrus pathogens.

Fungi as biocontrol agents

Biological control of postharvest citrus diseases by fungal antagonists is less developed compared with yeasts and bacteria. However, antagonistic fungi such as *Muscodor albus* and *Homoptera parasite* have shown a reduction in postharvest citrus fruit decay (Borras and Aguilar 1990; Benhamou 2004; Mercier and Smilanick 2005) (Table 1). Antagonistic fungi exhibit a broad spectrum in terms of disease control and volatile antimicrobial compounds (Mercier and Smilanick 2005; Verma *et al.* 2007). The fungus *M. albus*, a biofumigant that produces certain low-molecular-weight volatiles, has been used to fumigate whole rooms of lemons to control pathogens during storage. It is reported to be effective on green mould and sour rot (Mercier and Smilanick 2005). This fungus produces 28 organic volatile compounds that show some inhibitory effect against pathogenic fungi and bacteria (Strobel *et al.* 2001). This antagonism is due to the richness of its antimicrobial metabolites and to physiological conformation (Verma *et al.* 2007). The potential of fungal antagonists can be improved by continual improvement in isolation, formulation and application methods, particularly in the postharvest environment.

Table 1 Microbial antagonists used for the successful control of citrus postharvest diseases and their mode of action on citrus fruits

Microbial antagonist	Pathogen	Mode of action	References
Bacteria			
Pseudomonas syringae	*Penicillium digitatum*	Antibiosis, competition for nutrient	Bull *et al.* (1998)
	P. digitatum	Competition for nutrient and space	Smilanick and Denis-Arrue (1992)
	Penicillium spp	Antibiosis	Wilson and Chalutz (1989)
Pseudomonas glathei	*P. digitatum*	Competition for nutrient and space, induction of resistance	Huang *et al.* (1995)
Pseudomonas corrugata	*P. digitatum*	Antibiosis and competition for nutrients and space	Smilanick and Denis-Arrue (1992)
Pseudomonas fluorescens	*P. digitatum*	Antibiosis and competition for nutrients and space	Smilanick and Denis-Arrue (1992)
Enterobacter cloacae	*P. digitatum*	Competition for nutrient and space	Wilson and Chalutz (1989)
Bacillus subtilis	*P. digitatum*	Antibiosis	Singh and Deverall (1984)
	P. digitatum	Antibiosis	Leelasuphakul *et al.* (2008)
	P. digitatum *Geotrichum citri-aurantii*	Antibiosis	Yánez-Mendizábal *et al.* (2011)
Bacillus amyloliquefaciens	*Penicillium crustosum*	Production of volatile compounds	Arrebola *et al.* (2010)
Bacillus thuringiensis	*Guignardia citricarpa*	Antibiosis, induction of resistance	Lucon *et al.* (2010)
Serratia plymuthica	*Penicillium* spp	Antibiosis and competition for nutrients	Meziane *et al.* (2006)
Pantoea agglomerans	*Penicillium* spp	ND	Cañamás *et al.* (2008)
	P. digitatum	Triggers H2O2 production Triggers enzymatic activities	Torres *et al.* (2011)
Fungi			
Muscodor albus	*G. citri-aurantii* *P. digitatum*	Production of volatile compounds	Mercier and Smilanick (2005)
Aureobasidium pullulans	*P. digitatum*	Antibiosis	Liu, *et al.* (2007)
Verticillium lecanii	*P. digitatum*	Induction of resistance	Benhamou (2004)
Yeasts			
Pichia guilliermondii	*Penicillium italicum*	Competition for nutrient and space, directly parasitizing the pathogen	Arras *et al.* (1998)
	P. digitatum	Induction of resistance	Rodov *et al.* (1994)
Pichia anomala	*P. digitatum*	Competition for nutrient and space	Taqarort *et al.* (2008)
Pichia pastoris	*G. citri-aurantii*	Antibiosis	Ren *et al.* (2011)
Pichia membranaefaciens	*Penicillium* spp	Induction of resistance	Luo *et al.* (2012)
Candida saitoana	*P. italicum*	Competition for nutrient and space, direct parasitism	El-Ghaouth *et al.* (2000)
Candida famata	*P. digitatum*	Induction of resistance	Arras (1996)
Candida guilliermondii	*P. digitatum*	Induction of resistance	Arras (1996)
Candida oleophila	*P. digitatum*	Induction of resistance	Droby *et al.* (2002)
Candida sake	*P. digitatum*	ND	Droby *et al.* (1999)
Rhodotorula glutinis	*P. digitatum*	Competition for nutrient and space	Zheng *et al.* (2005)
Rhodosporidium paludigenum	*G. citri-aurantii*	Competition for nutrient and space	Liu *et al.* (2010)
Debaryomyces hansenii	*P. digitatum*	Competition for nutrient and space	Taqarort *et al.* (2008)
Cryptococcus laurentii	*P. italicum* *G. citri-aurantii*	Competition for nutrient and space	Liu *et al.* (2010)
A. pullulans	*Penicillium* spp	Competition for nutrient and space	Wilson and Chalutz (1989)
Kloeckera apiculata	*P. italicum*	Competition for nutrient and space	Long *et al.* (2006)
Wickerhamomyces anomalus	*P. digitatum*	Antibiosis	Platania *et al.* (2012)

ND, not determined.

Mode of action of antagonistic micro-organisms

Although considerable amount of research have been reviewed on the use of the microbial antagonists, few attempts have been made to study microbial interactions in fruit surface and wounds. This is due to difficulty in studying the complex interaction occurring between the pathogen, antagonist, host and other micro-organisms present on the fruit surface. Understanding the mechanism by which the biocontrol of fruit diseases occurs is critical to the eventual improvement and wider use of biocontrol methods. Several mechanisms, operating alone or in concert, are involved in antagonistic interactions in the fructoplane. Nutrient and space competition, antibiosis and parasitism are the major mechanisms involved. Additional mechanisms such as induced resistance, interference with pathogen-related enzymes and undoubtedly a number of still unknown mechanisms may complete the microbial arsenal (Sharma *et al.* 2009; Liu *et al.* 2013). Often, more than one mechanism is implicated, but in no case has a sole mechanism been found responsible for biological control (Janisiewicz and Korsten 2002; Liu *et al.* 2013). A good understanding of the relationships between pathogens, antagonistic micro-organisms, fruit and the environment is essential for the successful implementation of the biological control in the postharvest phase.

Antibiosis. Antibiotic production is one of the major modes of action of antagonists. This mechanism is found more in bacteria than in yeasts and in filamentous fungi. Bull *et al.* (1998) demonstrated that syringomycin produced by *Ps. syringae* controlled green mould on lemons and inhibited the growth of *G. citri-aurantii*, *P. digitatum* and *P. italicum*. Similarly, *Pseudomonas cepacia* was also found to be effective in controlling green mould in lemon by producing antibiotics (Smilanick and Denis-Arrue 1992). Moreover, *B. subtilis* and its antibiotics are considered to be potent biological control agents to suppress growth of *P. digitatum* in the postharvest protection of citrus (Leelasuphakul *et al.* 2008). However, the production of these antibiotics was not generally detected on the fruit; raising a doubt on the role of the antibiosis in postharvest diseases control which explains the fact that antibiosis may not comprise the entire mode of action of antagonists on citrus (Bull *et al.* 1998). Although antibiotic-producing bacteria have a potential to be used as biocontrol agents of postharvest diseases, importance is being given to the development of nonantibiotic-producing microbial antagonists for the control of postharvest diseases of fruits (El-Ghaouth *et al.* 2004). The possibility of rapid development of pathogen resistance towards antibiotic substances may be an obstacle in the practical use of antibiotic-producing micro-organisms for decay control.

Siderophores have also been reported to be produced by microbial antagonists. Pulcherrimin, a siderophore produced by the yeast *Metschnikowia pulcherrima*, has shown ability to reduce the growth of various postharvest fungal pathogens (Saravanakumar *et al.* 2008). Similarly, *Rhodotorula glutinis* controlled grey mould of apple by producing the siderophore rhodotorulic acid (Sansone *et al.* 2005). These studies established that competition for iron plays an important role in the biocontrol of pathogens by antagonistic micro-organisms.

Competition for nutrients and space. The nutrient sources in the peel of citrus fruit are frequently not sufficient for all micro-organisms, which makes the competition between pathogen and nonpathogens for nutrient resources or sites an important issue in biocontrol. Many investigations on different biocontrol systems have concluded that the successful competition of microbial antagonists with fruit-infecting pathogens for nutrients and space may be a possible mechanism of biocontrol (Wilson and Wisniewski 1989; Arras *et al.* 1998; Li *et al.* 2008; Liu *et al.* 2012). The nonpathogenic micro-organisms (especially yeasts) protect the surface of citrus fruits by rapid colonization of wounds and thereby exhausting the limited available substrates so that none are available for pathogen to growth. Several yeast species, *P. guilliermondii*, *C. saitoana*, *R. glutinis*, *Rhodosporidium paludigenum, K. apiculata*, *Hanseniaspora guilliermondii* and *Metschnikowia andauensis,* were reported to compete with citrus postharvest pathogens for nutrients and space (Arras *et al.* 1998; El-Ghaouth *et al.* 2000; Zheng *et al.* 2005; Long *et al.* 2006; Taqarort *et al.* 2008; Liu *et al.* 2010) (Table 1). The competition for nutrients and space is favoured by the attachment capability of antagonistic yeasts to pathogen hyphae. The attachment may enhance nutrient competition as well as interfere with the ability of the pathogen to initiate infection (El-Ghaouth *et al.* 2002). Currently, there are only fragmented data regarding the antagonist–pathogen interaction in terms of competitions for limiting nutrient essential for pathogenesis.

Direct parasitism. In direct parasitism, the pathogen is directly attacked by a specific microbial antagonist that kills it or its propagules. Parasitism depends on close contact and recognition between antagonist and pathogen, on the secretion of lytic enzymes and on the active growth of the parasite into the host (El-Ghaouth *et al.* 2002; Spadaro and Gullino 2004). It is often referred to as hyperparasitism or mycoparasitism when interactions involve a fungus. In the literature, the role of direct parasitism of the microbial antagonists in controlling postharvest pathogens of citrus fruit is less documented. Arras *et al.* (1998) showed that *P. guilliermondii* cells had the ability to attach to the hyphae of *P. italicum.* Also, the antagonistic yeast *C. saitoana* was found to attack *P. italicum* by direct parasitism (El-Ghaouth *et al.* 2000). The firm attachment of microbial antagonists to fungal pathogens, in conjunction with the enhanced activity of cell-wall degrading enzymes allowing invasion by mycoparasites, may have an important role in the biocontrol activity of antagonists.

Induced resistance. Several microbial antagonists elicit a wide range of defence responses termed induced resistance in citrus fruit (Borras and Aguilar 1990; Rodov *et al.* 1994; Droby *et al.* 2002; Benhamou 2004; Lucon *et al.* 2010). Droby *et al.* (2002) reported that the application of *C. oleophila* to surface wounds of grapefruit elicited systemic resistance against *P. digitatum.* They also demonstrated that the induction of pathogen resistance required viable yeast cells. Rodov *et al.* (1994) reported that *P. guilliermondii* induced resistance to green mould by eliciting the production of phytoalexins (e.g. scoparone and scopoletin). Similarly, Arras (1996) showed that scoparone accumulation could be 19 times higher when the antagonist *C. famata* was inoculated 24 h prior to *P. digitatum*, and only four times higher if inoculated 24 h after the pathogen. The accumulation of the phytoalexin scoparone was correlated with increased antifungal activity in the flavedo and resulted in enhanced resistance of the fruit to infection by *P. digitatum.* Recent studies have shown that microbial antagonists trigger a variety of cellular responses, such as activation of reactive oxygen species (ROS) and secretion of lytic enzymes such as b-1,3 glucanases and chitinases (Castoria *et al.* 2005; Chan *et al.* 2007; Friel *et al.* 2007; Macarisin *et al.* 2007; Xu and Tian 2008).

Although a causal connection between the accumulation of the host defence responses and bioprotection by microbial antagonists has not yet been clearly established, the occurrence of high levels of host antifungal compounds in protected tissue suggests their implication in disease resistance (El-Ghaouth *et al.* 2004).

Application methods of microbial antagonists

Infection of citrus fruit by pathogens can occur in the field prior to harvest thus it may be advantageous to apply microbial antagonists at this point, as well as in the postharvest phase. An important consideration for the application of antagonists at preharvest is their ability to colonize the surface of fruit both in the field and during storage and to persist on the fruit surface to maintain efficient control of decay (Ippolito and Nigro 2000; Cañamás *et al.* 2008). However, according to Sharma *et al.*(2009), this approach has still many limitations and does not provide commercially acceptable control of fruit diseases.

Unlike preharvest application, postharvest application of microbial antagonists is the most used and practical method for controlling postharvest diseases of citrus fruit (Sharma *et al.* 2009). In this case, microbial antagonists are applied either as postharvest spray, dip or drench applications (El-Ghaouth *et al.* 2001; Mercier and Smilanick 2005; Cañamás *et al.* 2008; Usall *et al.* 2008; Arrebola *et al.* 2010). Postharvest application of *Ps. syringae*, *Ps. cepacia*, *B. subtilis*, *Trichoderma viride* and *Debaryomyces hansenii* resulted in control of *P. digitatum* in citrus fruit (Singh and Deverall 1984; Wilson and Chalutz 1989; Borras and Aguilar 1990; Smilanick and Denis-Arrue 1992; Bull *et al.* 1998).

Biological control of citrus diseases with natural plant products

With consumer trends for natural alternatives to chemical-based fungicides and changes in legislation, the use of natural products such as plant extracts may provide a solution for both industry and consumers. Recently, attention has been paid towards the exploitation of higher plant products as novel botanical fungicides in citrus diseases management. More than 1340 plant species are known to be potential sources of antimicrobial compounds, and about 10 000 secondary plant metabolites have been chemically defined for their role as antimicrobials (Cowan 1999; Tripathi and Dubey 2003). Plant extracts have the advantage of being biodegradable, not phytotoxic, are GRAS to mammals. Therefore, higher plants can be exploited for the discovery of new natural fungicides, which can replace synthetic ones. Some phytochemicals of plant origin have been formulated as botanical pesticides and used successfully in integrated pest management programmes (Tripathi *et al.* 2004).

Use of essential oils

Essential oils are natural, volatile, complex compounds known for their antimicrobial, antioxidant and medicinal properties (Bakkali *et al.* 2008). The development of resistant strains of fungi against essential oils may be less likely as it is for many synthetic fungicides because several active components are often present in the final product and synergistic interactions may exist between the different components of the oils (Tripathi and Dubey 2003; Tripathi *et al.* 2004). Furthermore, most of the components of essential oils seem to have no specific cellular targets (Carson *et al.* 2002). The volatility, ephemeral nature and biodegradability of essential oil compounds may be especially advantageous for treatment of postharvest citrus diseases because only low levels of residues can be expected.

Plaza *et al.* (2004a) reported that thyme and cinnamon essential oils significantly reduced the incidence of green and blue moulds of citrus. Also, thyme oil was reported to control most postharvest citrus rots, such as green mould, blue mould and sour rot (Arras and Usai 2001; Liu *et al.* 2009b). Many studies (Table 2) have documented the antifungal effects of plant essential oils against citrus fruit pathogens (Chebli *et al.* 2003; Tripathi *et al.* 2004; Alilou *et al.* 2008; Du Plooy *et al.* 2009; Solaimani *et al.* 2009; Badawy *et al.* 2011). The antifungal activity of the essential oils suggests that they may be considered as a potential alternative to the synthetic fungicides for the control of postharvest citrus pathogens. However, despite their potent antifungal activity, commercial implementation of treatments with essential oils

Table 2 Plant extracts used for the control of citrus postharvest diseases

Plant species	Plant extract	Pathogen	References
Cymbopogon sp.	Aqueous extract	*Penicillium digitatum*	Abd-El-Khair and Hafez (2006)
Lantana sp.	Aqueous extract	*P. digitatum*	Abd-El-Khair and Hafez (2006)
Sanguisorba minor	Methanol extract	*P. digitatum* *Penicillium italicum*	Gatto *et al.* (2011)
Borago officinalis	Methanol extract	*P. digitatum* *P. italicum*	Gatto *et al.* (2011)
Sonchus oleraceus	Methanol extract	*P. digitatum* *P. italicum*	Gatto *et al.* (2011)
Thymus sp	Essential oil	*Geotrichum citri-aurantii*	Liu *et al.* (2009b)
Thymus capitatus	Essential oil	*P. digitatum*	Arras and Usai (2001)
Thymus vulgaris	Essential oil	*P. digitatum* *P. italicum* *Alternaria alternata* pv. *citri*	Fatemi *et al.* (2011)
Zataria multiflora	Essential oil	*P. digitatum* *P. italicum*	Solaimani *et al.* (2009)
Chrysanthemum	Essential oil	*G. citri-aurantii* *P. digitatum*	Chebli *et al.* (2003)
Cistus villosus	Aqueous extract Methanol extract	*G. citri-aurantii*	Talibi *et al.* (2012a) Talibi *et al.* (2012b)
Halimium antiatlanticum	Aqueous extract Methanol extract	*G. citri-aurantii*	Talibi *et al.* (2012a) Talibi *et al.* (2012b)
Halimium umbellatum	Aqueous extract	*G. citri-aurantii* *P. italicum*	Talibi *et al.* (2012a) Askarne *et al.* (2012)
	Methanol extract	*G. citri-aurantii*	Talibi *et al.* (2012b)
Mentha spicata	Essential oil	*P. digitatum*	Du Plooy *et al.* (2009)
Lippia scaberrima	Essential oil	*P. digitatum*	Du Plooy *et al.* (2009)
Allium sativum	Aqueous and ethanol extracts	*P. digitatum*	Obagwu and Korsten (2003)
Mentha arvensis	Essential oil	*P. italicum*	Tripathi *et al.* (2004)
Zingiber officinale	Essential oil	*P. italicum*	Tripathi *et al.* (2004)
Ocimum canum	Essential oil	*P. italicum*	Tripathi *et al.* (2004)
Acacia nilotica	Aqueous extract	*P. italicum*	Tripathi *et al.* (2002)
Punica granatum	Methanol extract	*P. digitatum*	Tayel *et al.* (2009)
Withania somnifera	Methanol extract	*P. digitatum*	Mekbib *et al.* (2007)
Acacia seyal	Methanol extract	*P. digitatum*	Mekbib *et al.* (2007)
Bubonium imbricatum	Essential oil	*P. digitatum*	Alilou *et al.* (2008)
Citrus sp.	Essential oil	*P. digitatum*	Badawy *et al.* (2011)
Ageratum conyzoides	Essential oil	*P. italicum*	Dixit *et al.* (1995)
Simmondsia chinensis	Oil emulsion	*P. italicum*	Ahmed *et al.* (2007)
Cinnamomumzeylanicum	Essential oil	*Penicillium* spp	Kouassi *et al.* (2012)
Parastrephia lepidophylla	Aqueous extract	*P. digitatum*	Sayago *et al.* (2012)

is strongly restricted in citrus because of problems related to potential phytotoxicity, intense sensory attributes or technological application as fumigants or in aqueous solutions (Palou *et al.* 2008).

Use of crude plant extracts

The preservative nature of some plant extracts has been known for centuries, and there has been renewed interest in the antimicrobial properties of extracts from aromatic plants. In recent years, several studies have been focused on screening of plant extracts for new antifungal compounds that can be used to control postharvest citrus diseases. Aqueous or organic solvent extracts of plants from different origins are sources of antifungal activity against citrus postharvest pathogens under different experimental conditions (Obagwu and Korsten 2003; Abd-El-Khair and Hafez 2006; Ameziane *et al.* 2007; Mekbib *et al.* 2007; Gatto *et al.* 2011; Talibi *et al.* 2011a; Askarne *et al.* 2012; Talibi *et al.* 2012a,b) (Table 2).

Besides the aqueous extracts, organic solvent extracts of several plants have been tested against citrus pathogens. Treatment of mandarin fruit with methanol extracts of *Cistus villosus*, *Halimium umbellatum* and *Ceratonia siliqua* successfully controlled the citrus sour rot (Talibi *et al.* 2012a,b; Askarne *et al.* 2013). Also of interest, methanol extracts from *Sanguisorba minor* showed good control of green mould (Gatto *et al.* 2011). Ameziane *et al.* (2007) showed that methanol extract of *C. villosus* is more active against *P. digitatum* and *G. citri-aurantii* than chloroform extracts. Thus, the nature of extraction influences the antifungal activity on the plants tested. This difference in biological activity is due to the polarity of each solvent, that is, the nature of the molecules extracted with each solvent. Methanol is a polar solvent, which can extract several compounds with antimicrobial activities such as alkaloids, triterpene glycosides, tannins, sesquiterpene lactones and phenolic compounds.The potential use of crude plant extracts to control postharvest citrus diseases requires a detailed examination of their biological activity and dispersion in fruit tissues and the development of a formulation which inhibits growth of pathogens without producing phytotoxic effects on fruit.

Use of natural products extracted from plants

Higher plants contain a wide spectrum of secondary substances such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins and sterols (Tripathi *et al.* 2004). Among the numerous natural plant products with potential antimicrobial activity are as follows: acetaldehyde, benzaldehyde, benzyl alcohol, ethanol, methyl salicylate, ethyl benzoate, ethyl formate, hexanal, (E)-2-hexenal, lipoxygenases, jasmonates, allicin, glucosinolates and isothiocyanates, etc. (Utama *et al.* 2002; Tripathi and Dubey 2003; Palou *et al.* 2008). Utama *et al.* (2002) demonstrated the efficacy of acetaldehyde, benzaldehyde, cinnamaldehyde, ethanol, benzyl alcohol, nerolidol and 2-nonanone as volatile fungitoxicants for the protection of citrus fruit against *P. digitatum.* Citral was reported to inhibit mycelial growth and spore germination of *P. digitatum* (Klieber *et al.* 2002). Also, citral has been highlighted as an active compound in citrus fruit against decay caused by *P. digitatum* (Fisher and Phillips 2008). Production of citral in the flavedo of citrus fruit has been described as a preformed defence mechanism against infection by *P. digitatum* (Rodov *et al.* 1995). Jasmonates (jasmonic acid and methyl jasmonate) have been found to be effective in postharvest control of *P. digitatum* either after natural or artificial inoculation of grapefruit (Droby *et al.* 1999). Exposure of fruit to jasmonates also effectively reduced chilling injury incidence after cold storage (Droby *et al.* 1999). As they are naturally occurring compounds and are given in low doses, jasmonates may provide a more environment-friendly means of reducing the use of synthetic chemicals. A naturally occurring compound isolated from the flavedo tissue of grapefruit (*Citrus paradisi*) identified as 7-geranoxy coumarin exhibited antifungal activity against *P. italicum* and *P. digitatum* during *in vitro* and *in vivo* tests (Agnioni *et al.* 1998) (Table 3).

Table 3 Natural compounds tested against citrus postharvest pathogens

Compound	Causal agent	References
Benzaldehyde	*Penicillium digitatum*	Wilson and Wisniewski (1989)
Cinnamaldehyde	*P. digitatum*	Wilson and Wisniewski (1989)
Acetaldehyde vapour	*P. digitatum*	Prasad and Stadelbacher (1973)
Heptanol	*Geotrichum citri-aurantii*	Suprapta *et al.* (1997)
Decanol	*G. citri-aurantii*	Suprapta *et al.* (1997)
Geraniol	*G. citri-aurantii*	Suprapta *et al.* (1997)
Citronellol	*G. citri-aurantii*	Suprapta *et al.* (1997)
Citral	*G. citri-aurantii*	Suprapta *et al.* (1997) Klieber *et al.* (2002) Zhou *et al.* (2014)
	P. digitatum	Fisher and Phillips (2008)
Thymol	*P. digitatum*	Jafarpour and Fatemi (2012)
Menthol	*P. digitatum*	Jafarpour and Fatemi (2012)
7-geranoxy coumarin	*P. digitatum* *Penicillium italicum*	Agnioni *et al.* (1998)
Jasmonic acid	*P. digitatum*	Droby *et al.* (1999)
Methyl jasmonate	*P. digitatum*	Droby *et al.* (1999)
Nerolidol	*P. digitatum*	Droby *et al.* (1999)
2-nonanone	*P. digitatum*	Droby *et al.* (1999)
Kaempferol	*P. italicum*	Tripathi *et al.* (2002)

Mode of action of plant extracts

As the exploitation of natural plant products to protect the postharvest decay of citrus fruit is in its infancy, there is little information about their mechanism of action. Nevertheless, some data address their modes of action. Considering the large number of bioactive chemicals in plant extracts, it is most likely that their antimicrobial activity is not attributable to one specific mechanism but to diverse modes of action (Carson *et al.* 2002). Droby *et al.* (1999) reported that jasmonates suppress the development of *P. digitatum* in grapefruit. This control might be due to the induction of host resistance responses (Droby *et al.* 1999). Also, Tripathi and Dubey (2003) reported that jasmonates play an important role as signal molecules in plant defence responses against pathogen attack. The same authors showed that essential oils play a role in plant defence mechanisms against phytopathogenic micro-organisms, and the synergism between their different components reduces the development of resistant races of fungi. Also methanol extracts of *Withania somnifera* and *Acacia seyal* controlled green mould by a stimulatory effect on host defence mechanisms (Lanciotti *et al.* 2004; Mekbib *et al.* 2007). These defence mechanisms resulted in: (i) synthesis of cell wall that could serve as a physical and biological barrier to invading pathogens or (ii) an increase in the total soluble phenolic compound concentration of orange peels (Mekbib *et al.* 2007). Phenolic compounds are known to alter membrane functionality of pathogens (Lanciotti *et al.* 2004). However, more investigations on the mode of action of such plant products are required before their recommendation for the control of citrus postharvest diseases.

Application methods of plant extracts and criteria for selecting a good product

In *in vivo* trials, the efficacy of postharvest treatments of plant extracts on citrus fruits depended on their method of application. The incorporation of essential oils into fruit coatings primarily applied to retain moisture is gaining popularity (Du Plooy *et al.* 2009). Essential oil of *Simmondsia chinensis* (jojoba oil) was applied by Ahmed *et al.* (2007) as a coating for 'Valencia' oranges. They effectively maintained fruit quality for up to 60 days (Ahmed *et al.* 2007). Also Du Plooy *et al.* (2009) showed that the advantage of using coatings amended with essential oils, rather than vapour, is that there is closer contact between the essential oils and fruit surfaces, allowing exposure of each fruit to similar concentrations of inhibitor over a longer period. Another method of application of botanicals in controlling citrus postharvest diseases is the immersion of citrus fruits in plant solutions. Essential oil of Shiraz thyme showed antifungal activity against *P. digitatum* only when dip applied (Solaimani *et al.* 2009). The same authors reported that the dipping method was significantly better than spray method on control of green mould. Keeping in view the merits of the botanicals as postharvest fungitoxicants and to ensure proper application of plant extracts, the products which are found efficacious during *in vivo* application must meet the following conditions: (i) the product should be effective even after treatments of short duration; (ii) the treatment should not have an effect on quality parameters such as acidity, flavour and aroma and (iii) the lowest suitable dose of the treatments for practical application should be utilized (Tripathi and Dubey 2003).

Control of citrus postharvest diseases by food additives and GRAS compounds

Antifungal compounds that leave low or nondetectable residues in the citrus fruit are actively sought in research programmes. Organic and inorganic salts are widely used in the food industry; they are common food additives for leavening, pH control, taste and texture modifications (Smilanick *et al.* 1999). These compounds have a broad spectrum of activity against bacteria and fungi and are GRAS compounds for many applications, by European and North America regulations. In addition to their consistent antimicrobial activity, they are inexpensive, readily available, with favourable safety profile for humans and the environment and suitable for commercial postharvest handling practices (El-Mougy *et al.* 2008; Deliopoulos *et al.* 2010) (Table 4).

Use of GRAS compounds and food additives

Among food preservatives, potassium sorbate has been evaluated for the control of citrus green and blue moulds and sour rot (Kitagawa and Kawada 1984; Hall 1988; Palou *et al.* 2002; El-Mougy *et al.* 2008; Smilanick *et al.* 2008; Youssef *et al.* 2012a,b). This compound, classified as a minimal risk active ingredient and exempt from residue tolerances, is more appropriate for application as an aqueous solution. However, it is not a popular control agent because its low efficacy, and it was reported to delay, rather than stop, green mould infections (Smilanick *et al.* 2008). Sodium benzoate and benzoic acid are known for their bactericidal and bacteriostatic properties

Table 4 Salts and food additives used for the control of citrus postharvest diseases

Name of salt	Causal agent	References
Acetic acid	*Penicillium digitatum*	Sholberg (1998)
Formic acid	*P. digitatum*	Sholberg (1998)
Propionic acid	*P. digitatum*	Sholberg (1998)
Sorbic acid	*Geotrichum citri-aurantii*	Kitagawa and Kawada (1984)
Benzoic acid	*G. citri-aurantii* *Penicillium italicum* *P. digitatum*	El-Mougy *et al.* (2008)
Boric acid	*G. citri-aurantii*	Talibi *et al.* (2011b)
Potassium sorbate	*P. digitatum*	Smilanick *et al.* (2008), D'Aquino *et al.* (2013)
	G. citri-aurantii	El-Mougy *et al.* (2008)
	P. italicum	El-Mougy *et al.* (2008)
Sodium carbonate	*P. italicum*	
	P. digitatum	Plaza *et al.* (2004c)
Sodium bicarbonate	*P. digitatum*	Smilanick *et al.* (1999)
	G. citri-aurantii	Smilanick *et al.* (2008)
	P. italicum	Palou *et al.* (2001)
Sodium benzoate	*G. citri-aurantii* *P. italicum* *P. digitatum*	El-Mougy *et al.* (2008)
	P. digitatum	Hall (1988)
Sodium salicylate	*G. citri-aurantii*	Talibi *et al.* (2011b)
Sodium propionate	*P. digitatum*	Hall (1988)
Sodium ethylparaben	*P. italicum*	Moscoso-Ramírez *et al.* (2013)
Calcium chloride	*P. digitatum*	Droby *et al.* (1997)
Calcium polysulfide	*P. digitatum* *G. citri-aurantii*	Smilanick and Sorenson (2001)

and have the advantage of being nontoxic and tasteless (El-Mougy *et al.* 2008). Their effect on postharvest citrus diseases was reported against sour rot, green and blue moulds of stored citrus fruit (Hall 1988; El-Mougy *et al.* 2008). A comparison among the inhibitory effects of various food additives and low-toxicity chemicals against *P. digitatum* and *P. italicum* showed that potassium sorbate and sodium benzoate were the most effective on oranges and lemons (Palou *et al.* 2002). To extend the shelf life of fresh fruit, many other salt compounds are actually used in many countries, aiming at the destruction of the pathogens or inhibition of their growth. Certain compounds, such as sodium bicarbonate or sodium carbonate, have been highly successful in controlling green mould (Smilanick *et al.* 1999). Smilanick *et al.* (2008) reported that sodium bicarbonate reduced the incidence of citrus sour rot. Youssef *et al.* (2014) demonstrated that both sodium carbonate and bicarbonate exert a direct antifungal effect on *P. digitatum* and induce citrus fruit defence mechanisms to postharvest decay. Moreover, these treatments pose a minimal risk of phytotoxicity to the fruit and can be a useful tool in the management of fungicide-resistant isolates, which have become particularly problematic (Smilanick *et al.* 1999). A significant reduction in incidence of *P. digitatum* and *P. italicum* was noted in the case of oranges treated with ammonium molybdate and sodium molybdate (Palou *et al.* 2002). Besides these salts, other compounds such as orthophosphoric acid, sodium propionate, calcium polysulfide, calcium chloride, EDTA, sodium salicylate and boric acid have been evaluated for the control of citrus green or blue moulds or sour rot and reduced the incidence and severity of these diseases (Hall 1988; Droby *et al.* 1997; El-Mougy *et al.* 2008; Talibi *et al.* 2011b) (Table 4).

Mode of action of salt compounds

Although many researchers have focused on the control of postharvest diseases of citrus fruit by the application of salt compounds, the mechanisms by which salts inhibit micro-organisms are not well understood. The various modes of action of salt compounds are membrane disruption, inhibition of essential metabolic functions, stresses on pH homeostasis through the accumulation of anions within the cell and the activation of defence mechanisms in fruit (Smilanick *et al.* 2005; Youssef *et al.* 2014). Bicarbonates are effective growth inhibitors of various phytopathogenic fungi *in vitro*. Most citrus fungal pathogens grow better in acidic to neutral conditions than in alkaline conditions. The principal mode of action of the bicarbonate ion is through its buffering capacity sustaining an alkaline environment. When this happens, pathogens, such as *P. digitatum* which require an acidic environment, expend more energy on fungal acid production than hyphal extension and therefore growth may be inhibited (Pelser and Eckert 1977). Inhibition of *G. citri-aurantii*, *P. digitatum* and *P. italicum* by sorbic acid and its salts may be caused by alteration of cell membrane function and cell transport function, inhibition of enzymes and protein synthesis, and uncoupling of oxidative phosphorylation in mitochondria (El-Mougy *et al.* 2008). The pH of the bicarbonate and carbonate solutions is important for the control of postharvest citrus diseases, because it directly affects the germination of conidia (Pelser and Eckert 1977) and influences the virulence of pathogens through their colonization of host tissue (Smilanick *et al.* 2005). However, we and other workers showed that pH alone cannot explain the inhibitory effect of these compounds (Palmer *et al.* 1997; Smilanick *et al.* 1999; Talibi *et al.* 2011b; Youssef *et al.* 2014).

The effectiveness of calcium against *P. digitatum* on grapefruit could be due to its direct effects on host tissue by making cell walls more resistant to pathogen penetration. Most of the calcium that penetrates into the host tissue seems to accumulate in the middle lamella region of the cell wall. These cations form bonds between adjacent pectic acids or between pectic acids and other polysaccharides, forming cross-bridges which make the cell walls less accessible to the action of pectolytic enzymes of the pathogen (Droby *et al.* 1997). Ammonium molybdate affects metabolic processes in several organisms (Bodart *et al.* 1999). The basis of its biological activity was reported to be its ability to inhibit acid phosphatase which interferes with phosphorylation and dephosphorylation (Mukhopadhyay *et al.* 1988), one of the most important processes of cell regulation (Hunter 1995).

Use of combined strategy to control postharvest citrus diseases

Different disease management strategies applied both at pre- and postharvest stages have been integrated to provide more effective disease control than possible with a single approach. Several lines of evidence suggest that the combination of microbial antagonists with other alternative control methods can be a promising approach to overcome some of the drawbacks in biocontrol activity (Huang *et al.* 1995; Droby *et al.* 1998; El-Ghaouth *et al.* 2000; Arras *et al.* 2002; Janisiewicz and Korsten 2002; Porat *et al.* 2002; Plaza *et al.* 2004b,c; Zhang *et al.* 2004) (Table 5). The combination of microbial antagonists with heat (Porat *et al.* 2002), GRAS compounds (Usall *et al.* 2008) and UV-C (Stevens *et al.* 1997) produced a synergic effect and was superior to all the treatments alone in controlling green and blue moulds. Such combined

Table 5 Combination of biocontrol antagonists with other control methods

Antagonist	Combined with	Disease controlled	References
Combination with physical control methods			
Candida oleophila	Hot water	Green mould	Porat *et al.* (2002)
	Ultraviolet light-C		D'hallewin *et al.* (2004)
Pseudomonas glathei	Heat treatment	Green mould	Huang *et al.* (1995)
Bacillus subtilis	Hot water	Green mould	Obagwu and Korsten (2003)
Pantoea agglomerans	Heat treatment	Green mould	Plaza *et al.* (2004c)
Debaryomyces hansenii	Ultraviolet light-C	Green mould	Stevens *et al.* (1997)
Combination with low levels of conventional fungicides			
C. oleophila	Thiabendazole	Stem-end rot	Brown and Chambers (1996)
		Penicillium rots	Droby *et al.* (1998)
Pichia guilliermondii	Imazalil	Stem-end rot	Arras *et al.* (2002)
	Thiabendazole	Penicillium rots	
Kloeckera apiculata	Carbendazim	Blue mould	Long *et al.* (2006)
Combination with food additives and other salts			
C. oleophila	Sodium bicarbonate	Green mould	Porat *et al.* (2002)
Bacillus subtilis	Sodium bicarbonate	Green mould	Obagwu and Korsten (2003)
Pseudomonas syringae	Sodium bicarbonate	Green mould	Smilanick *et al.* (1999)
	Sodium carbonate		
Pantoea agglomerans	Sodium carbonate	Green mould	Usall *et al.* (2008)
	Sodium bicarbonate		Teixidó *et al.* (2001)
Cryptococcus laurentii	Sodium bicarbonate	Green mould	Zhang *et al.* (2004)
Pichia guilliermondii	Calcium chloride	Green mould	Droby *et al.* (1997)
Candida saitoana	Glycolchitosan	Green mould	El-Ghaouth *et al.* (2000)
	2-Deoxy-D-glucose		El-Ghaouth *et al.* (2001)
Kluyveromyces marxianus	Sodium bicarbonate	Green mould	Geng *et al.* (2011)

treatments can be easily implemented on a commercial scale in many citrus packinghouses because they are compatible with existing facilities and postharvest handling practices. In general, five objectives may be pursued by the integration of two or more treatments: additive or synergistic effects to increase the effectiveness or the persistence of individual treatments; complementary effects to combine preventive and curative activities; to delay the development of fungicide-resistant isolates, to control fungicide-resistant isolates already present within packinghouses; and to facilitate a reduction in fungicide rates in order to minimize fruit residues and chemical costs (Palou *et al.* 2008; Smilanick *et al.* 2008). For example, combination of biocontrol agents with salts or food additives, low levels of conventional fungicides or physical control treatments is possible to improve the action of biological control agents.

Combination of microbial antagonists with other control methods

As previously mentioned, the main shortcoming of the use of microbial antagonists has been inconsistency in their performance, especially when used as a stand-alone product to replace synthetic fungicides. Furthermore, as infection of citrus fruit occurs either prior to harvest or during harvesting and processing, microbial antagonists are expected to display both a protective and curative activity comparable to that observed with synthetic fungicides. However, the biocontrol agents often fail to control previously established infections (Ippolito and Nigro 2000; Zheng *et al.* 2005). The combination of biological control with other control methods is one of the most promising means of establishing effective integrated disease management strategies. Several approaches have been evaluated to enhance the biocontrol properties of antagonists (Table 5).

Combination of salts and food additives with physical control treatments

Salts and food additives are more effective when combined with curing and hot water treatments. Sorbate potassium was reported to control postharvest sour rot when it was applied as a hot water solution (Kitagawa and Kawada 1984; Smilanick *et al.* 2008). Plaza *et al.* (2004c) reported that dipping fruits in a sodium carbonate solution following a curing treatment satisfactorily reduced incidence of green and blue moulds during subsequent long-term storage. Besides heat treatments, salts are applied with wax coatings, a critical operation in citrus fruit packinghouses. Youssef *et al.* (2012a,b) reported that wax mixed with sodium bicarbonate, potassium carbonate and potassium sorbate significantly reduced the incidence of postharvest green and blue moulds.

Conclusion

From this review, it appears that some significant progress has been made towards the biological and integrated control of postharvest diseases of citrus fruit. Some biofungicides are already on the market in a few countries and will probably become more widely available as they are registered in more areas. Other microbial antagonists should reach the market soon. Postharvest conditions provide an ideal niche for microbial antagonists as they are less subject to sudden weather changes and are often equipped with a sophisticated climate control systems. However, so far, only a few products with high biocontrol potential have been made available on a commercial scale. With intensive research being carried out in various laboratories, the possibility of identifying potent microbes and developing suitable biocontrol products for commercial marketing appears to be bright. On the other hand, it is unrealistic to assume that microbial antagonists have the same fungicidal activity as fungicides. Improved postharvest usage strategies of microbial antagonists include integration with other low-risk treatments to optimize performance while allowing identification of methods that reduce the use of conventional synthetic fungicides for the control of postharvest diseases of citrus fruit. In the development of these new strategies, emphasis should be placed on minimizing human health risks and environmental toxicity. Research should provide appropriate tools (microbial antagonists, natural substances, GRAS compounds, etc.. . .) to tailor a complete postharvest citrus diseases management strategy.

Conflict of interest

No conflict of interest declared.

References

Abd-El-Khair, H. and Hafez, O. (2006) Effect of aqueous extracts of some medicinal plants in controlling the green mould diseases and improvement of stored "Washington" navel orange quality. *Appl Sci Res* **2**, 664–674.

Agnioni, A., Cabras, P., Dhallewin, G., Pirisi, F., Reniero, F. and Schirra, M. (1998) Synthesis and inhibitory activity of 7-geranoxy coumarin against *Penicillium* species in citrus fruits. *Phytochemistry* **47**, 1521–1525.

Ahmed, D.M., El Shami, S. and El Mallah, M.H. (2007) Jojoba oil as a novel coating for exported Valencia orange fruit part 1: the use of trans (isomerized) jojoba oil. *Am Eurasian J Agric Environ Sci* **2**, 173–181.

Alilou, H., Akssira, M., Hassani, L., Chebli, B., EL Hakmoui, A., Mellouki, F., Rouhi, R., Boira, H. *et al.* (2008) Chemical composition and antifungal activity of *Bubonium imbricatum* volatile oil. *Phytopathol Mediterranea* **47**, 3–10.

Ameziane, N., Boubaker, H., Boudyach, E.H., Msanda, F., Jilal, A. and Ait Ben Aoumar, A. (2007) Antifungal activity of Moroccan plants against citrus fruit pathogens. *Agron Sustain Dev* **27**, 273–277.

Arras, G. (1996) Mode of action of an isolate of *Candida famata* in biological control of *Penicillium digitatum* in orange fruits. *Postharvest Biol Technol* **8**, 191–198.

Arras, G. and Usai, M. (2001) Fungitoxic activity of 12 essential oils against four postharvest citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *J Food Prot* **64**, 1025–1029.

Arras, G., De Cicco, V., Arru, S. and 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.

Arras, G., Scherm, B. and Migheli, Q. (2002) Improving biocontrol activity of *Pichia guilliermondii* against postharvest decay of oranges in commercial packing-houses by reduced concentrations of fungicides. *Biocontrol Sci Tech* **12**, 547–553.

Arrebola, E., Sivakumar, D. and Korsten, L. (2010) Effect of volatile compounds produced by *Bacillus* strains on postharvest decay in citrus. *Biol Control* **53**, 122–128.

Askarne, L., Talibi, I., Boubaker, H., Boudyach, E., Msanda, F., Saadi, B., Serghini, M. and Ait Ben Aoumar, A. (2012) *In vitro* and *in vivo* antifungal activity of several Moroccan plants against *Penicillium italicum*, the causal agent of citrus blue mold. *Crop Prot* **40**, 53–58.

Askarne, L., Talibi, I., Boubaker, H., Boudyach, E., Msanda, F., Saadi, B. and Ait Ben Aoumar, A. (2013) Use of Moroccan medicinal plant extracts as botanical fungicide against citrus blue mould. *Lett Appl Microbiol* **56**, 37–43.

Badawy, F.M.I., Sallam, M.A.N., Ibrahim, A. and Asran, M. (2011) Efficacy of some essential oils on controlling green mold of orange and their effects on postharvest quality parameters. *Plant Pathol J* **10**, 168–174.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological effects of essential oils-a review. *Food Chem Toxicol* **46**, 446–475.

Baudoin, A.B.A.M. and Eckert, J.W. (1985) Influence of preformed characteristics of lemon peel on susceptibility to *Geotrichum candidum*. *Physiol Plant Pathol* **26**, 151–163.

Benhamou, N. (2004) Potential of the mycoparasite, *Verticillium lecanii*, to protect citrus fruit against *Penicillium digitatum*, the causal agent of green mold: a comparison with the effect of chitosan. *Phytopathology* **94**, 693–705.

Bodart, J.-F., Béchard, D., Bertout, M., Rousseau, A., Gannon, J., Vilain, J.-P. and Flament, S. (1999) Inhibition of protein tyrosine phosphatases blocks calcium-induced activation of metaphase II-arrested oocytes of *Xenopus laevis*. *FEBS Lett* **457**, 175–178.

Borras, A.D. and Aguilar, R.V. (1990) Biological control of *Penicillium digitatum* by *Trichoderma viride* on postharvest citrus fruits. *Int J Food Microbiol* **11**, 179–183.

Boubaker, H., Saadi, B., Boudyach, E.H. and Ait Benaoumar, A. (2009) Sensitivity of *Penicillium digitatum* and *P. italicum* to imazalil and thiabendazole in Morocco. *Plant Pathol J* **4**, 152–158.

Brown, G.E. (1988) Efficacy of guazatine and iminoctadine for control of postharvest decays of oranges. *Plant Dis* **72**, 906–908.

Brown, G.E. and Chambers, M. (1996) Evaluation of biological products for the control of postharvest diseases of Florida citrus. *Proc Fla State Hortic Soc* **109**, 278–282.

Bull, C., Wadsworth, M., Sorensen, K., Takemoto, J., Austin, R. and Smilanick, J. (1998) Syringomycin E produced by biological control agents controls green mold on lemons. *Biol Control* **12**, 89–95.

Caccioni, D.R.L., Guizzardi, M., Biondi, D.M., Renda, A. and Ruberto, G. (1998) Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *Int J Food Microbiol* **43**, 73–79.

Cañamás, T.P., Viñas, I., Usall, J., Torres, R., Anguera, M. and Teixidó, N. (2008) Control of postharvest diseases on citrus fruit by preharvest applications of biocontrol agent *Pantoea agglomerans* CPA-2: part II. Effectiveness of different cell formulations. *Postharvest Biol Technol* **49**, 96–106.

Carson, C.F., Mee, B.J. and Riley, T.V. (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob Agents Chemother* **46**, 1914–1920.

Castoria, R., Morena, V., Caputo, L., Panfili, G., De Curtis, F. and De Cicco, V. (2005) Effect of the biocontrol yeast *Rhodotorula glutinis* strain LS11 on patulin accumulation in stored apples. *Phytopathology* **95**, 1271–1278.

Chalutz, E. and Wilson, C. (1990) Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*. *Plant Dis* **74**, 134–137.

Chan, Z., Qin, G., Xu, X., Li, B. and Tian, S. (2007) Proteome approach to characterize proteins induced by antagonist yeast and salicylic acid in peach fruit. *J Proteome Res* **6**, 1677–1688.

Chebli, B., Achouri, M., Idrissi Hassani, L. and Hmamouchi, M. (2003) Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea Pers*: Fr. *J Ethnopharmacol* **89**, 165–169.

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

D'Aquino, S., Fadda, A., Barberis, A., Palma, A., Angioni, A. and Schirra, M. (2013) Combined effects of potassium sorbate, hot water and thiabendazole against green mould of citrus fruit and residue levels. *Food Chem* **141**, 858–864.

Deliopoulos, T., Kettlewell, P.S. and Hare, M.C. (2010) Fungal disease suppression by inorganic salts: a review. *Crop Prot* **29**, 1059–1075.

D'hallewin, G., Arras, G., Venditti, T., Rodov, V. and Ben-Yehoshua, S. (2004) Combination of ultraviolet-c irradiation and biocontrol treatments to control decay caused by *Penicillium digitatum* in 'Washington navel' orange fruit. *Acta Hort* **682**, 2007–2012.

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

Droby, S., Wisniewski, M., Cohen, L., Weiss, B., Touitou, D., Eilam, Y. and Chalutz, E. (1997) Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue, and biocontrol activity of *Pichia guilliermondii*. *Phytopathology* **87**, 310–315.

Droby, S., Cohen, L., Daus, A., Weiss, B., Horev, B., Chalutz, E., Katz, H., Keren-Tzur, M. *et al.* (1998) Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. *Biol Control* **12**, 97–101.

Droby, S., Porat, R., Cohen, L., Weiss, B., Shapiro, B., Philosoph-Hadas, S. and Meir, S. (1999) Suppressing green mold decay in grapefruit with postharvest jasmonate application. *J Am Soc Hort Sci* **124**, 184–188.

Droby, S., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E. and Porat, R. (2002) Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. *Phytopathology* **92**, 393–399.

Droby, S., Wisniewski, M., Macarisin, D. and Wilson, C. (2009) Twenty years of postharvest biocontrol research: is it time for a new paradigm? *Postharvest Biol Technol* **52**, 137–145.

Du Plooy, W., Regnier, T. and Combrinck, S. (2009) Essential oil amended coatings as alternatives to synthetic fungicides in citrus postharvest management. *Postharvest Biol Technol* **53**, 117–122.

El-Ghaouth, A., Smilanick, J.L. and Wilson, C.L. (2000) Enhancement of the performance of *Candida saitoana* by the addition of glycochitosan for control of postharvest decay of apple and citrus fruit. *Postharvest Biol Technol* **19**, 249–253.

El-Ghaouth, A., Smilanick, J.L., Brown, G.E., Ippolito, A. and Wilson, C.L. (2001) Control of decay of apple and citrus fruits in semicommercial tests with *Candida saitoana* and 2-deoxy-d-glucose. *Biol Control* **20**, 96–101.

El-Ghaouth, A., Wilson, C., Wisniewski, M., Droby, S., Smilanick, J.L. and Korsten, L. (2002) Biological control of postharvest diseases of fruits and vegetables. *Appl Mycol Biotechnol* **2**, 219–238.

El-Ghaouth, A., Wilson, C. and Wisniewski, M. (2004) Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases of fruit and vegetables. In *Diseases of Fruits and Vegetables: Volume II* ed. Naqvi, S.A.M.H. pp. 511–535. the Netherlands: Springer.

El-Mougy, N.S., El-Gamal, N.G. and Abd-El-Kareem, F. (2008) Use of organic acids and salts to control postharvest diseases of lemon fruits in Egypt. *Archiv Phytopathol Plant Prot* **41**, 467–476.

Fatemi, S., Jafarpour, M. and Eghbalsaied, S. (2011) Study of the effect of Thymus vulgaris and hot water treatment on storage life of orange (*Citrus sinensis* CV. Valencia). *J Med Plants Res* **6**, 968–971.

Fisher, K. and Phillips, C. (2008) Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends Food Sci Technol* **19**, 156–164.

Friel, D., Gomez Pessoa, M.G., Vandenbol, M. and Jijakli, M.H. (2007) Separated and simultaneous disruptions of two exo-b-1, 3-glucanase genes decrease the biocontrol efficiency of *Pichia anomala* (strain K). *Mol Plant Microbe Interact* **20**, 371–379.

Gatto, M.A., Ippolito, A., Linsalata, V., Cascarano, N.A., Nigro, F., Vanadia, S. and Di Venere, D. (2011) Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biol Technol* **61**, 72–82.

Geng, P., Chen, S., Hu, M., Rizwan-ul-Haq, M., Lai, K., Qu, F. and Zhang, Y. (2011) Combination of *Kluyveromyces marxianus* and sodium bicarbonate for controlling green mold of citrus fruit. *Int J Food Microbiol* **151**, 190–194.

Hall, D.J. (1988) Comparative activity of selected food preservatives as citrus postharvest fungicides. *Proc Fla State Hort Soc* **101**, 184–187.

Huang, Y., Deverall, B.J. and Morris, S.C. (1995) Postharvest control of green mould on oranges by a strain of *Pseudomonas glathei* and enhancement of its biocontrol by heat treatment. *Postharvest Biol Technol* **5**, 129–137.

Hunter, T. (1995) Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell* **80**, 225–236.

Ippolito, A. and Nigro, F. (2000) Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Prot* **19**, 715–723.

Ismail, M. and Zhang, J. (2004) Post-harvest citrus diseases and their control. *Outlooks Pest Manage* **15**, 29–35.

Jafarpour, M. and Fatemi, S. (2012) Post harvest treatments on shelf life of sweet orange Valencia. *J Med Plants Res* **6**, 2117–2124.

Janisiewicz, W. (1987) Postharvest biological control of blue mold on apples. *Phytopathology* **77**, 481–485.

Janisiewicz, W.J. and Korsten, L. (2002) Biological control of postharvest diseases of fruits. *Annu Rev Phytopathol* **40**, 411–441.

Kanetis, L., Förster, H. and Adaskaveg, J.E. (2007) Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. *Plant Dis* **91**, 1502–1511.

Kitagawa, H. and Kawada, K. (1984) Effect of sorbic acid and potassium sorbate on the control of sour rot of citrus fruits. *Proc Fla State Hort Soc* **97**, 133–135.

Klieber, A., Scott, E. and Wuryatmo, E. (2002) Effect of method of application on antifungal efficacy of citral against postharvest spoilage fungi of citrus in culture. *Aust Plant Pathol* **31**, 329–332.

Kouassi, K.H.S., Bajji, M. and Jijakli, H. (2012) The control of postharvest blue and green molds of citrus in relation with essential oil–wax formulations, adherence and viscosity. *Postharvest Biol Technol* **73**, 122–128.

Ladaniya, M.S. (2008) Postharvest diseases and their management. In *Citrus Fruit: Biology, Technology and Evaluation*, pp. 417–449. San Diego: Academic Press.

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. and Gardini, F. (2004) Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends Food Sci Technol* **15**, 201–208.

Leelasuphakul, W., Hemmanee, P. and Chuenchitt, S. (2008) Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. *Postharvest Biol Technol* **48**, 113–121.

Li, B.Q., Zhou, Z.W. and Tian, S.P. (2008) Combined effects of endo- and exogenous trehalose on stress tolerance and biocontrol efficacy of two antagonistic yeasts. *Biol Control* **46**, 187–193.

Lima, G., Ippolito, A., Nigro, F. and Salerno, M. (1997) Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. *Postharvest Biol Technol* **10**, 169–178.

Liu, X., Wang, J., Gou, P., Mao, C., Zhu, Z.R. and Li, H. (2007) In vitro inhibition of postharvest pathogens of fruit and control of gray mold of strawberry and green mold of citrus by aureobasidin A. *Int J Food Microbiol* **119**, 223–229.

Liu, B., Qiao, H., Huang, L., Buchenauer, H., Han, Q., Kang, Z. and Gong, Y. (2009a) Biological control of take-all in wheat by endophytic *Bacillus subtilis* E1R-j and potential mode of action. *Biol Control* **49**, 277–285.

Liu, X., Wang, L., Li, Y., Li, H., Yu, T. and Zheng, X. (2009b) Antifungal activity of thyme oil against *Geotrichum citri-aurantii in vitro* and *in vivo*. *J Appl Microbiol* **107**, 1450–1456.

Liu, X., Fang, W., Liu, L., Yu, T., Lou, B. and Zheng, X. (2010) Biological control of postharvest sour rot of citrus by two antagonistic yeasts. *Lett Appl Microbiol* **51**, 30–35.

Liu, J., Wisniewski, M., Droby, S., Norelli, J., Hershkovitz, V., Tian, S. and Farrell, R. (2012) Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* following sublethal oxidative stress exposure. *FEMS Microbiol Ecol* **80**, 578–590.

Liu, J., Sui, Y., Wisniewski, M., Droby, S. and Liu, Y. (2013) Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int J Food Microbiol* **167**, 153–160.

Long, C.A., Deng, B.X. and Deng, X.X. (2006) Pilot testing of *Kloeckera apiculata* for the biological control of postharvest diseases of citrus. *Ann Microbiol* **56**, 13–17.

Lucon, C., Guzzo, S., de Jesus, C., Pascholati, S. and de Goes, A. (2010) Postharvest harpin or *Bacillus thuringiensis* treatments suppress citrus black spot in 'Valencia' oranges. *Crop Prot* **29**, 766–772.

Luo, Y., Zeng, K. and Ming, J. (2012) Control of blue and green mold decay of citrus fruit by *Pichia membranefaciens* and induction of defense responses. *Sci Hortic (Amsterdam)* **135**, 120–127.

Macarisin, D., Cohen, L., Eick, A., Rafael, G., Belausov, E., Wisniewski, M. and Droby, S. (2007) *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. *Phytopathology* **97**, 1491–1500.

Mekbib, S., Regnier, T. and Korsten, L. (2007) Control of *Penicillium digitatum* on citrus fruit using two plant extracts and study of their mode of action. *Phytoparasitica* **35**, 264–276.

Mercier, I. and Smilanick, J.L. (2005) Control of green mold and sour rot of stored lemon by biofumigation with *Muscodor albus*. *Biol Control* **32**, 401–407.

Meziane, H., Gavriel, S., Ismailov, Z., Chet, I., Chernin, L. and Hofte, M. (2006) Control of green and blue mould on orange fruit by *Serratia plymuthica* strains IC14 and IC1270 and putative modes of action. *Postharvest Biol Technol* **39**, 125–133.

Moscoso-Ramírez, P.A., Montesinos-Herrero, C. and Palou, L. (2013) Control of citrus postharvest penicillium molds with sodium ethylparaben. *Crop Prot* **46**, 44–51.

Mukhopadhyay, N.K., Saha, A.K., Lovelace, J.K., Silva, R., Sacks, D.L. and Glew, R.H. (1988) Comparison of the protein kinase and acid phosphatase activities of five species of Leishmania. *J Eukaryot Microbiol* **35**, 601–607.

Nunes, C.A. (2012) Biological control of postharvest diseases of fruit. *Eur J Plant Pathol* **133**, 181–196.

Obagwu, J. and Korsten, L. (2003) Control of citrus green and blue molds with garlic extracts. *Eur J Plant Pathol* **109**, 221–225.

Palmer, C.L., Horst, R.K. and Langhans, R.W. (1997) Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. *Plant Dis* **81**, 1432–1438.

Palou, L., Smilanick, J.L., Usall, J. and Viñas, I. (2001) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Dis* **85**, 371–376.

Palou, L., Usall, J., Smilanick, J.L., Aguilar, M.J. and Vinas, I. (2002) Evaluation of food additives and low toxicity compounds as alternative chemicals for the control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. *Pest Manag Sci* **58**, 459–466.

Palou, L., Smilanick, J.L. and Droby, S. (2008) Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Posthar Rev* **4**, 1–16.

Pelser, P.D.T. and Eckert, J. (1977) Constituents of orange juice that stimulate the germination of conidia of *Penicillium digitatum*. *Phytopathology* **67**, 747–754.

Platania, C., Restuccia, C., Muccilli, S. and Cirvilleri, G. (2012) Efficacy of killer yeasts in the biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*). *Food Microbiol* **30**, 219–225.

Plaza, P., Torres, P., Usall, J., Lamarca, N. and Vinas, I. (2004a) Evaluation of the potential of commercial post-harvest application of essential oils to control citrus decay. *J Hort Sci Biotechnol* **79**, 935–940.

Plaza, P., Usall, J., Smilanick, J.L., Lamarca, N. and Vinas, I. (2004b) Combining *Pantoea agglomerans* (CPA-2) and curing treatments to control established infections of *Penicillium digitatum* on lemons. *J Food Prot* **67**, 781–786.

Plaza, P., Usall, J., Torres, R., Abadias, M., Smilanick, J.L. and Vinas, I. (2004c) The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. *Pest Manag Sci* **60**, 815–821.

Porat, R., Daus, A., Weiss, B., Cohen, L. and Droby, S. (2002) Effects of combining hot water, sodium bicarbonate and biocontrol on postharvest decay of citrus fruit. *J Hort Sci Biotechnol* **77**, 441–445.

Prasad, K. and Stadelbacher, G.J. (1973) Control of post harvest decay of fresh raspberries by acetaldehyde vapor. *Plant Dis* **57**, 795–797.

Ren, X., Kong, Q., Wang, H., Yu, T., Zhou, W. and Zheng, X. (2011) Biocontrol of fungal decay of citrus fruit by *Pichia pastoris* recombinant strains expressing cecropin A. *Food Chem* **131**, 796–801.

Rodov, V., Ben-Yehoshua, S., Fang, D., D'hallewin, G. and Castia, T. (1994) Accumulation of phytoalexins scoparone and scopoletin in citrus fruits subjected to various postharvest treatments. *Acta Hort* **381**, 517–525.

Rodov, V., Ben-Yehoshua, S., Fang, D.Q., Kim, J.J. and Ashkenazi, R. (1995) Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. *J Agric Food Chem* **43**, 1057–1061.

Sansone, G., Rezza, I., Calvente, V., Benuzzi, D. and Sanz de Tosetti, M.I. (2005) Control of Botrytis cinerea strains resistant to iprodione in apple with rhodotorulic acid and yeasts. *Postharvest Biol Technol* **35**, 245–251.

Saravanakumar, D., Ciavorella, A., Spadaro, D., Garibaldi, A. and Gullino, M.L. (2008) *Metschnikowia pulcherrima* strain MACH1 outcompetes *Botrytis cinerea*, *Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. *Postharvest Biol Technol* **49**, 121–128.

Sayago, J.E., Ordoñez, R.M., Kovacevich, L.N., Torres, S. and Isla, M.I. (2012) Antifungal activity of extracts of extremophile plants from the Argentine Puna to control citrus postharvest pathogens and green mold. *Postharvest Biol Technol* **67**, 19–24.

Sharma, R., Singh, D. and Singh, R. (2009) Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. *Biol Control* **50**, 205–221.

Sholberg, P. (1998) Fumigation of fruit with short-chain organic acids to reduce the potential of postharvest decay. *Plant Dis* **82**, 689–693.

Singh, V. and Deverall, B. (1984) *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. *Trans Br Mycol Soc* **83**, 487–490.

Smilanick, J. and Denis-Arrue, R. (1992) Control of green mold of lemons with *Pseudomonas* species. *Plant Dis* **76**, 481.

Smilanick, J. and Sorenson, D. (2001) Control of postharvest decay of citrus fruit with calcium polysulfide. *Postharvest Biol Technol* **21**, 157–168.

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

Smilanick, J., Mansour, M., Margosan, D., Gabler, F.M. and Goodwine, W. (2005) Influence of pH and NaHCO3 on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Dis* **89**, 640–648.

Smilanick, J.L., Mansour, M.F., Gabler, F.M. and Sorenson, D. (2008) Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Biol Technol* **47**, 226–238.

Solaimani, B., Ramezani, S., Rahemi, M. and Saharkhiz, M.J. (2009) Biological control of postharvest disease caused by *Penicillium digitatum* and *P. italicum* on stored citrus fruits by Shiraz Thyme essential oil. *Adv Environ Biol* **3**, 249–254.

Spadaro, D. and Gullino, M.L. (2004) State of the art and future prospects of the biological control of postharvest fruit diseases. *Int J Food Microbiol* **91**, 185–194.

Spotts, R.A., Cervantes, L.A. and Facteau, T.J. (2002) Integrated control of brown rot of sweet cherry fruit with a preharvest fungicide, a postharvest yeast, modified atmosphere packaging, and cold storage temperature. *Postharvest Biol Technol* **24**, 251–257.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Pusey, P.L., Igwegbe, E.C.K., Kabwe, K., Mafolo, Y. *et al.* (1997) Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. *Biol Control* **10**, 98–103.

Strobel, G.A., Dirkse, E., Sears, J. and Markworth, C. (2001) Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. *Microbiology* **147**, 2943.

Suprapta, D.N., Arai, K. and Iwai, H. (1997) Effects of volatile compounds on arthrospore germination and mycelial growth of *Geotrichum candidum* citrus race. *Mycoscience* **38**, 31–35.

Talibi, I., Amkraz, N., Askarne, L., Msanda, F., Saadi, B., Boudyach, E.H., Boubaker, H., Bouizgarne, B. *et al.* (2011a) Antibacterial activity of Moroccan plants extracts against *Clavibacter michiganensis* subsp. michiganensis, the causal agent of tomatoes' bacterial canker. *J Med Plants Res* **5**, 4332–4338.

Talibi, I., Askarne, L., Boubaker, H., Boudyach, E.H. and Ait Ben Oumar, A. (2011b) *In vitro* and *in vivo* Screening of organic and inorganic slats to control postharvest citrus sour rot caused by *Geotrichum candidum*. *Plant Pathol J* **10**, 138–145.

Talibi, I., Askarne, L., Boubaker, H., Boudyach, E.H., Msanda, F., Saadi, B. and Ait Ben Oumar, A. (2012a) Antifungal activity of some Moroccan plants against *Geotrichum candidum*, causal agent of postharvest citrus sour rot. *Crop Prot* **35**, 41–46.

Talibi, I., Askarne, L., Boubaker, H., Boudyach, E.H., Msanda, F., Saadi, B. and Ait Ben Aoumar, A. (2012b) Antifungal activity of Moroccan medicinal plants against citrus sour rot agent *Geotrichum candidum*. *Lett Appl Microbiol* **55**, 155–161.

Taqarort, N., Echairi, A., Chaussod, R., Nouaim, R., Boubaker, H., Benaoumar, A.A. and Boudyach, E. (2008) Screening and identification of epiphytic yeasts with potential for biological control of green mold of citrus fruits. *World J Microbiol Biotechnol* **24**, 3031–3038.

Tayel, A., El-Baz, A., Salem, M. and El-Hadary, M. (2009) Potential applications of pomegranate peel extract for the control of citrus green mould. *J Plant Dis Plant Prot* **6**, 252–256.

Teixidó, N., Usall, J., Palou, L., Asensio, A., Nunes, C. and Viñas, I. (2001) Improving control of green and blue molds of oranges by combining *Pantoea agglomerans* (CPA-2) and sodium bicarbonate. *Eur J Plant Pathol* **107**, 685–694.

Torres, R., Teixidó, N., Usall, J., Abadias, M., Mir, N., Larrigaudiere, C. and Viñas, I. (2011) Anti-oxidant activity of oranges after infection with the pathogen *Penicillium digitatum* or treatment with the biocontrol agent *Pantoea agglomerans* CPA-2. *Biolo Control* **57**, 103–109.

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

Tripathi, P. and Dubey, N. (2003) 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., Banerji, R. and Chansouria, J. (2004) Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits. *World J Microbiol Biotechnol* **20**, 317–321.

Usall, J., Smilanick, J., Palou, L., Denis-Arrue, N., Teixidó, N., Torres, R. and Viñas, I. (2008) Preventive and curative activity of combined treatments of sodium carbonates and *Pantoea agglomerans* CPA-2 to control postharvest green mold of citrus fruit. *Postharvest Biol Technol* **50**, 1–7.

Utama, I.M.S., Wills, R.B.H., Ben-yehoshua, S. and Kuek, C. (2002) In vitro efficacy of plant volatiles for inhibiting the growth of fruit and vegetable decay microorganisms. *J Agric Food Chem* **50**, 6371–6377.

Verma, M., Brar, S.K., Tyagi, R., Surampalli, R. and Valéro, J. (2007) Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochem Eng J* **37**, 1–20.

Wilson, C.L. and Chalutz, E. (1989) Postharvest biological control of *Penicillium* rots of citrus with antagonistic yeasts and bacteria. *Sci Hortic* **40**, 105–112.

Wilson, C.L. and Wisniewski, M.E. (1989) Biological control of postharvest diseases of fruits and vegetables: an emerging technology. *Annu Rev Phytopathol* **27**, 425–441.

Xu, X.B. and Tian, S.P. (2008) Reducing oxidative stress in sweet cherry fruit by *Pichia membranaefaciens*: a possible mode of action against *Penicillium expansum*. *J Appl Microbiol* **105**, 1170–1177.

Yánez-Mendizábal, V., Usall, J., Viñas, I., Casals, C., Marín, S., Solsona, C. and Teixidó, N. (2011) Potential of a new strain of *Bacillus subtilis* CPA-8 to control the major postharvest diseases of fruit. *Biocontrol Sci Tech* **21**, 409–426.

Youssef, K., Ligorio, A., Nigro, F. and Ippolito, A. (2012a) Activity of salts incorporated in wax in controlling postharvest diseases of citrus fruit. *Postharvest Biol Technol* **65**, 39–43.

Youssef, K., Ligorio, A., Sanzani, S.M., Nigro, F. and Ippolito, A. (2012b) Control of storage diseases of citrus by pre- and postharvest application of salts. *Postharvest Biol Technol* **72**, 57–63.

Youssef, K., Sanzani, S.M., Ligorio, A., Ippolito, A. and Terry, L.A. (2014) Sodium carbonate and bicarbonate treatments induce resistance to postharvest green mould on citrus fruit. *Postharvest Biol Technol* **87**, 61–69.

Zhang, H.Y., Fu, C.X., Zheng, X.D., He, D., Shan, L.J. and Zhan, X. (2004) Effects of *Cryptococcus laurentii* (Kufferath) Skinner in combination with sodium bicarbonate on biocontrol of postharvest green mold decay of citrus fruit. *Bot Bull Acad Sin* **45**, 159–164.

Zheng, X.D., Zhang, H.Y. and Sun, P. (2005) Biological control of postharvest green mold decay of oranges by *Rhodotorula glutinis*. *Eur Food Res Technol* **220**, 353–357.

Zhou, H., Tao, N. and Jia, L. (2014) Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citri-aurantii*. *Food Control* **37**, 277–283.