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Emerging Non-Conventional Technologies for Control of Post Harvest Diseases of Perishables

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ABSTRACT

Considerable amounts of fruits and vegetables are lost to spoilage after harvest. This loss can range from 10-50% depending on the commodity and country. Presently, synthetic chemicals are the primary means of controlling post harvest diseases of fruits and vegetables. Public concern over food safety, however, enunciated interest to find out the effective alternatives to chemical pesticides to control post harvest diseases of perishables. The ultimate aim of recent research in this area has been the development and evaluation of various alternative control strategies to reduce dependency on synthetic fungicides. Currently several promising biological approaches that include the application of microbial antagonists (fungi, bacteria, yeasts), the natural plant based antimicrobial substances (volatile aromatic compounds, acetic acid, jasmonates, glucosinolates, essential oils, plant extracts and propolis), the antimicrobial substances from soil (fusapyrone and deoxyfusapyrone) and the natural animal-based antimicrobial substances like chitosan have been advanced to curb the menaces of post harvest diseases in perishables. Compounds that activate host plant defense responses potentially offer socio-environmentally sound alternative methods for disease control. Combination of the above complementary techniques could well lead to effective control of post harvest diseases. The techniques and practice of using all these non-conventional alternatives is still in its infancy as compared to chemical treatments but the results and progress in this area during the past decade has been remarkable.

Keywords: antagonists, chitosan, deoxyfusapyrone, essential oils, fusapyrone, glucosinolates, propolis

CONTENTS

INTRODUCTION

Considerable amounts of perishables are lost after harvest. Mechanical, physiological and phytopathological factors are responsible for post harvest losses in fruits and vegetables. Even a small loss can be very expensive because of the accumulated cost of growing, harvesting and storing these high value commodities. In developing countries this

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loss can range from 10-50% depending on the commodity, production area and season.

Chemical treatment has proved to be a promising control measure for product protection, but is permitted in only a few species. Public opinion demands a reduction in the use of synthetic chemicals due to the direct exposure to the treated commodities, carcinogenicity, teratogenicity, high and acute residual toxicity, long degradation period, phytotoxicity and off odour effects, environmental pollution and other side effects on humans. Further the effectiveness of the post harvest chemical treatment decreases with the appearance of resistant strains. The intense selection pressure of pesticide residues on the treated perishable commodities is responsible for the rapid spread of resistance among pathogens (Vinas *et al.* 1991). Fruits and vegetables have specially been placed under scrutiny because of being contaminated with potentially carcinogenic compounds such as 'Alar' (Chalutz and Wilson 1990). All these issues have stimulated the need to search some alternative sources for controlling post harvest rots of perishables. At present biocontrol by the application of microbial antagonists (fungi, bacteria, yeasts), natural plant-based antimicrobial substances (volatile aromatic compounds, acetic acid, jasmonates, glucosinolates, essential oils, plant extracts and propolis), antimicrobial substances from soil (fusapyron and deoxyfusapyrone) and natural animal-based antimicrobial substances like chitosan have emerged as the latest non-conventional technologies and as a new hope for the present scenario.

APPLICATION OF ANTAGONISTIC MICROORGANISMS

Over the past 15 years the use of antagonistic microorganisms has emerged as an effective non-conventional biocontrol strategy to combat major post harvest decay of perishables (Wilson *et al.* 1999; Janisiewicz and Korsten 2002). An alternate to chemical pesticides microbial antagonists are being used to control post harvest decay of fruits and vegetables. The major advantages of using antagonistic microorganisms in post harvest management of fruits and vegetables are: controlled and stable environmental conditions in storage rooms, which favors antagonist survival; ability to apply antagonists directly to targeted area (fruit); ease in manipulation of the post harvest system; and exemption from tolerance of the registered antagonists (Janisiewicz 2003). The success of some of the microbial antagonists in laboratories and large-scale studies has stimulated the interest of several workers in the development of biological products for post harvest application.

Antagonistic microorganisms

A number of microorganisms (bacteria, yeasts and fungi) which effectively control post harvest pathogens have been identified for post harvest control (Wilson and Wisniewski 1989; Wisniewski and Wilson 1992). It has been found that a bacterial population lives and reproduces inside healthy fruits and acts as an antagonist. In the case of pears *Bacillus pumilus* controlled grey mold on artificially inoculated pears with *B. cinerea* and storing at 20°C for at least 9 days (Mari *et al.* 1996a). It was also reported that holding bacteria treated fruits at 20°C for 24 hours before cold storage improved the efficiency of *B. pumilus* against grey mold. To control grey mold in tomato *Bacillus amyloquefaciens* was effective on both mature green and red tomatoes (Mari *et al.* 1996b). It was also reported that chilled injured mature green tomatoes were more susceptible to *B. cinerea* and on these fruits *B. amyloquefaciens* completely controlled the pathogen if the treatment was given immediately after storage at 2°C. In pilot experiments with wounded fruits dipped in the *B. amyloquefaciens* and *B. cinerea* suspension, the development of decay was effectively inhibited. *Pichia guilliermondii*, *P. anomala*, *Cryptococcus laurentii* and *Sporobolomyces roseus* are some examples of yeast antagonists and proved most prominent in biocontrol of a number of fruit pathogenic microbes. The strains of *Trichoderma harzianum* (Guizzardi *et al.* 1995) and *Trichoderma pseudokoningii* (Tronsmo and Raa 1977) are the fungal antagonists against *Monilinia laxa* in stone fruits and *B. cinerea* in apples.

Source of natural antagonists

The surface of fruits and vegetables is an excellent source of naturally occurring antagonists against their own post harvest decay. Healthy perishables in the orchard and storage are an ideal place where a disease must be expected but if it does not occur (Baker and Cook 1974) it may be due to already existing microflora on the surface of perishables which act as a source of effective antagonist against post harvest pathogens of perishables (Janisiewicz 1988). Therefore, the most abundant and most desirable source for isolating antagonists against post harvest fruit pathogens is fructoplanes. Phylloplane could be a source of antagonists as it may share part of the resident microflora of fruits as well as contain other microorganisms (Wisniewski *et al.* 1991; Koomen and Jeffries 1993; Smilanick *et al.* 1999). Soil is another abundant and diverse source of antagonists. *Bacillus subtilis* B_3 isolated from peach roots and which inhibited *Monilinia fructicola* on agar plates, is a very effective strain for the biocontrol of brown rot of peaches (Pusey and Wilson 1984). B_3 was the first extensively studied strain for the biocontrol of post harvest disease. The early success in biocontrol of brown rot of peach with this strain generated interest in biocontrol of post harvest diseases of fruits.

For the isolation of microorganisms growing efficiently on the substrate, a number of enrichment procedures have been used viz. agar plates containing apple juice that were seeded with fruit washings (Janisiewicz 1988a), fruit wounds treated with fruit washings and incubated for several days (Weller 1988), freshly made wounds on apples in the orchard that were exposed to colonization by fruit-associated microbial flora from one to four weeks before harvest (Janisiewicz 1991) and from apple juice culture resulting from seeding diluted apple juice with the orchard colonized wounds and repeated reinoculation to fresh apple juice.

Probable mechanisms of biocontrol

The common mechanism of biocontrol among antagonists appears to be competition for nutrients and space (Mari *et al.* 1996a). Some other mechanisms are also suggested to be involved such as production of antifungal metabolites (Janisiewicz *et al.* 1991), direct parasitism, and induced resistance. Induced resistance may be associated with a reduction of pathogen enzyme activity (Zimand *et al.* 1996). More than one mechanism has been found to be implicated in biocontrol. Competition for nutrients and space seems to play a major role, however appropriate methods are lacking to separate the various mechanisms of action (Janisiewicz and Kortsen 2002). Promising results are expected by biological sensors (Lindow *et al.* 2001) and cylinder-well tests to study antagonist-pathogen interactions (Janisiewicz *et al.* 2000).

The use of antibiotic-producing bacteria has been abandoned (Wisniewiski and Wilson 1992) and the use of yeasts and non-antibiotic–producing bacteria as antagonists appear to be quite promising although the mechanism has not been fully explored. Competition for nutrients and space as well as direct parasitism are the two probable mechanisms by which yeasts act as an antagonist, e.g. the US-7 stain of *Pichia guilliermondii* is an antagonist of *P. digitatum* in grape fruit and *B. cinerea* in apples. In addition to the ability of yeasts cells to grow very quickly and thus to remove nutrients and space from the pathogen (Droby *et al.* 1989), they are also able to produce hydrolytic enzymes (β-1,3-glucanase) capable of attaching to the cell walls of *B. cinerea* and extracellular polymers that appear to have antifungal activity (Droby *et al.* 1993). When a fungal antagonist like *Trichoderma* is used as an antagonist, mycoparasitism is the most probable mechanism.

The antifungal effectiveness of an antagonist can be increased by addition of substances such as calcium salts or sugar analogs and calcium chloride that improves biological control activity of the yeast *P. guilliermondii* (Droby *et al.* 1997), while 2-deoxy-D-glucose, in combination with a mutant strain of *Sporobolomyces roseus*, decreases the concentration of the antagonist required for biocontrol of blue mod on apples (Janisiewicz 1994).

Qualities of a potent antagonist

An ideal antagonist should be one which must be as potent as the chemical means to control and that at a low concentration could effectively control a range of pathogens. It should be non-toxic to mammals and other animals, able to survive in adverse conditions (Janisiewicz 1988) and must be easy to culture and amenable to growth on an inexpensive medium and must be prepared in an easily distributable form with adequate shelf life. The other important qualities which a potent antagonist for post harvest diseases control must have are it must be genetically stable, it should be resistant to pesticides, it must be compatible with other treatments (physical and chemical) and must be non pathogenic against the host plant.

Some commercial antagonists

Some microbial antagonists have been patented and registered (Mendelson *et al.* 1994). Currently *Pseudomonas syringae* (BioSave 100^{TM} and 110^{TM}) for the control of *Geotricum candidum* on pome and citrus fruits and *Candida oleophila* (Aspire^{TM}) for the control of *Penicillium* decay on citrus have been registered by Ecogen Inc. in the USA (Shachnal *et al.* 1996). Other antagonists such as *Bacillus subtilis* (Avogreen) have been registered in South Africa for the control of pre and post harvest diseases of avocado (Janisiewicz and Korsten 2002) and *Cryptococcus albidus* (Yield Plus) for the control of post harvest diseases of apples and pears.

Limitations

However, the use of antagonist organisms has its own limitations, which may restrict its use under some circumstances. Their potency is generally limited due to their limited spectrum of activity and efficiency under some environmental conditions, specificity against various diseases of fruits and lack of eradicative activity (Janisiewicz 2003). The decrease in efficacy and lack of consistency are the hurdles in the way when this methodology is applied as stand-alone treatment under commercial conditions. Martin and Bull (2002) concluded that while single microbial inoculants may provide some control for specific diseases of strawberry, they cannot provide the broad spectrum control needed to replace methyl bromide. Although many useful biocontrol agents were first identified through *in vitro* inhibition tests (i.e. evaluating inhibition of a target pathogen on an agar medium) several researchers have reported no correlation between *in vitro* inhibition tests and field performance of biocontrol agents. For example, Burr *et al.* (1996) found no correlation between the ability of bacteria and yeasts to inhibit *Venturia inequalis in vitro* and the ability to control apple scab. A frequent criticism of antagonists use as biocontrol measure is that the extent of control may differ with varying environmental parameters. For example, Haung *et al* (2000) attributed inconsistent performance of biocontrol agents (antagonistic microorganisms) against white mold of beans between years of field testing to environmental differences. Further, the most important factor limiting commercial interest in using antagonists is the cost of production for most biocontrol agents (Fravel *et al.* 1999).

NATURAL PLANT-BASED ANTIMICROBIAL SUBSTANCES

Higher plants are a rich source of novel natural substances that can be used to develop environmentally safe methods. In recent years, interest in natural substances has increased and numerous studies on the biocidal activities of a wide range of secondary metabolites viz. phenols, flavonoids, quinines, tannins, essential oils, alkaloids, saponins and sterols produced by plants have been reported. Such plant chemicals may be exploited for their different biological properties (Poulev *et al.* 2003; Isman 2006). Botanicals, because of their natural origin are biodegradable and mostly do not leave toxic residues or byproducts to contaminate the environment.

Volatile aromatic components

Fruit and vegetables have a number of constitutive and inducible volatile, aromatic and flavour compounds that are antimicrobial and such compounds have not been fully explored as biological control agents for post harvest diseases (Culter *et al.* 1986). These aromatic and flavour components are generally produced by fruits during ripening and provide resistance to the fruits at the post harvest stage. The flavour compounds are secondary metabolites having unique properties of volatility, and fat and low-water solubility. Being volatile they are easily adsorbed and may be proved very useful in post harvest protection. Many such compounds are harmless for mammalian systems and there is less chance of off odours in treated commodities. Most of the volatiles are effective at very low concentrations. As potential fungicides, their natural occurrence as part of the diet, their ephemeral nature, and their biodegradability suggest low toxic residue problems. Such compounds could be extracted and applied to other harvested perishables. Some of the volatile aromatic components viz. acetaldehyde, six carbon (C_6) aldehydes, benzaldehyde, hexenel and hexanal are discussed under the following headings.

Acetaldehyde

It has been found that a number of volatiles are produced by peaches having strong fungicidal activity (Wilson *et al.* 1987). Acetaldehyde has been used as a fumigant to control the green peach aphid on head lettuce (Stewart *et al.* 1980). In response to high CO_2 storage conditions strawberries show heavy rot resistance due to the production of high levels of acetaldehyde and ethyl acetate (Shaw 1969). Vapours of acetaldehyde have been used to control *Botrytis cinerea* (Prasad and Stadelbacher 1973). Acetaldehyde has been tested against the fungi like *B. cineria* and *Rhizopus stolonifer* causing rot to strawberry fruits (Avissar and Pesis 1991). In addition, acetaldehyde has also been reported to inhibit post harvest microorganisms such as *Erwinia carotovora*, *Pseudomonas fluorescens*, *Monilinia fructicola* (Aharoni and Stadelbacher 1973), *Penicillium* spp. (Stadelbacher and Prasad 1974) and various species of yeasts (Barkai-Golan and Ahroni 1976) commonly found on fruit and vegetables.

Six carbon (C_6) aldehydes

Six carbon (C_6) aldehydes have been found to inhibit the hyphal growth of *Alternaria alternata* and *B. cinerea* (Hamilton-Kemp *et al.* 1992). These aldehydes, with or without double bonds, are dominant compounds released by plant material through the lipoxygenase pathway after tissue damage (Vick and Zimmerman 1987). They are also important precursors for the formation of C_6 alcohols and C_6 esters, which are among the most abundant volatile components in apple, pear and banana, and contribute to typical fruity odours (Paillard 1986, 1990). This suggests that hexenal and similar aldehydes may be used as antifungal agents with fruit such as pears, strawberries, banana, pineapples and melons. *In vitro* spore germination and mycelial growth assay against *P. expansum* showed a consistent fungicidal activity of *trans*-2-hexenal at 9.4 µl/l and carvacrol at 3.4 µl/l, while the other aldehydes viz. hexanal, (-) carvone, *p*-anisaldehyde, eugenol and 2-nananone exhibited a progressively lower inhibition. *Trans*-2-hexenal was the best inhibitor of conidial germinations at 9.4 µl/l, while carvacrol was the best inhibitor of mycelial growth of *P. expansum* at 3.4 µl/l (Neri *et al.* 2006a). *In vivo* potential for the control of blue mold of pears was shown by *trans*-2-hexanal vapours at 12.5 µl/l at 20ºC on 'Conference' pears (Neri *et al.* 2006b). Use of these aldehydes in packaging of highly processed products of these commodities also seems to be a possible future option. However, practical doses of these compounds still need to be worked out, particularly in relation to any mammalian toxicity that might occur.

Benzaldehyde

Benzaldehyde has been used in the laboratory to fumigate peaches and to protect them against *Rhizopus* rot. It totally inhibits spore germination of *B. cinerea* at 25µl/l and germination of *M. fructicola* at 125 µg/l (Wilson *et al.* 1987). The aldehydes (benzaldehyde along with some other plant volatiles e.g. acetaldehyde, cinnamaldehyde, ethaniol, benzyl alcohol) were found to be the strongest growth inhibitors and the most lethal to the fungal spores and mycelia of fruit and vegetable pathogens viz. *P. digitatum*, *R. stolonifer*, *Colletotrichum musae* and bacterial cells of *Erwinia caratovora* during *in vitro* trials. The average minimum inhibitory concentrations (MICs) of aldehydes that were germicidal to decay microorganisms were 0.28, 0.49, and 0.88 mmol per Petri dish, for cinnamaldehyde, benzaldehyde, and acetaldehyde, respectively. Ethanol also inhibited growth completely, but the MIC, which was 14.6 mmol per Petri dish, was significantly higher than those of the aldehydes. The ketones (nerolidol, and 2-nonanone) tended to be effective only on *P. digitatum* and *C. musae* (Utma *et al.* 2002).

Hexenal and hexanal

(*E*)-2-hexenal (C_6H_{12} O) and hexanal ($C_6H_{12}O$) are two different volatile flavour compounds. Hexenal vapours have a number of attributes that may be important in consumer demand for more natural measures for fruit diseases with fewer toxic residues. Hexenal vapour inhibited hyphal growth of apple slices (Song *et al.* 1996). This raises the possibility of developing a system for treating apple slices with hexenal in modified atmospheres and packages. Archbold *et al.* (1999) showed (*E*)-2-hexenal to be an efficient fumigant in controlling mold on 'Crimson Seedless' table grapes. (*E*)-2-Hexenal, was found to be strongly antifungal in nature and its *in vitro* and *in vivo* activity has been reported by a number of workers against *B. cinerea* (Hamilton-Kemp *et al.* 1992; Fallik *et al.* 1998). The effect of *trans*-2-hexenal on the control of blue mold disease (*P. expansum*), in reduction of patulin content and on fruit quality improvement of 'Conference' pears was evaluated and greater reduction of decay was obtained by treatment at 12.5 µl/l at 20ºC for 24 or 48 h after inoculation (Neri *et al.* 2006b).

Acetic acid

The shelf life of some fruits can be extended through surface sterilization by a suitable non-toxic edible chemical. Fumigation with acetic acid offers a promising method for surface sterilization of a wide range of perishables. It is a metabolic intermediate that occurs naturally in many fruits (Nursten 1970). The inhibitory effect of acetic acid on microorganisms is greater not only due to pH alone but also the undissociated acetic acid can penetrate the microbial cell to exert its toxic effect (Banwart 1981). There are several advantages in using acetic acid fumigation. It is a natural compound found throughout the biosphere, posing little or no residual hazard. It inhibits many species of bacteria, yeasts and molds. Low concentrations i.e. 2.0 or 4.0 mg/l of acetic acid in air has been found to be extremely effective for control of *B. cinerea* conidia on apple ('Red Delicious) fruit (Sholberg and Gaunce 1995). Fumigation with acetic acid protected grapes from spoilage for up to 2 months in modified atmosphere packing (MAP) at 0ºC, and presents a possible alternative for extending the shelf life of grapes (Molys *et al.* 1996). Acetic acid has been shown to be an effective fumigant for commercial use on apricot and plums (Liu *et al.* 2002), grapes (Sholberg *et al.* 1996) and sweet cherries (Sholberg 1998; Chu *et al.* 1999, 2001). The use of acetic aid and vinegar are the better choice in most cases because it does not have an objectionable odor and has a long history of use on food (Sholberg 1998; Sholberg *et al.* 2000). The use of acetic acid would be inexpensive as compared to other fumigants such as acetaldehyde, and can be used in relatively low concentrations. It can be used to treat the perishables in air-tight storage rooms or containers. In addition being a natural compound it has been regarded as a safe compound in the USA (Chiechester and Tanner 1981) and, therefore does not require rigorous registration procedure for use on food commodities.

Jasmonates

The term jasmonates include jasmonic acid (JA) and methyl jasmonate (MJ). These are naturally occurring plant growth regulators that are widely distributed in the plant kingdom, and are known to regulate various aspects of plant development and responses to environmental stresses (Sembdner and Parthier 1993; Creelman and Mullet 1995, 1997). Jasmonates are a class of olypines derived from oxygenase-dependent oxidation of fatty acids. Jasmonates play an important role as signal molecules in plant defense responses against pathogenic attack. JA accumulates in plant tissues or in cell cultures treated with elicitors of plant defense mechanisms (Gundlach *et al.* 1992; Doares *et al.* 1995; Nojiri *et al.* 1996). Several jasmonates have been shown to activate genes coding antifungal proteins such as thionin (Andresen *et al.* 1992), osmotin (Xu *et al.* 1994) a novel ribosome inactive protein (Chaudhry *et al.* 1994) and several other genes involved in phytotoxin biosynthesis (Creelman *et al.* 1992; Gundlach *et al.* 1992).The exposure of the diced pineapple to a MJ emulsion at a concentration of 10^{-4} M for 5 min in a sealed container decreased microbiological growth. MJ as vapor or as dip did not affect the firmness or the colour of the fruit (Martinez and Harper 2005). Droby *et al.* (1999) found that post harvest application of jasmonates reduced decay caused by grey mold, *P. digitatum* either after natural or artificial inoculation of 'Marsh Seedless' grapefruit. Free radical scavenging capacities of strawberries and black berries treated with MJ and allyl isothiocyanate (AITC) reduced the severity of decay in both strawberries and black berries during storage at 10°C as compared to control (Chanjirakul *et al.* 2007).

The volatility of MJ allows treatments to be applied without immersing fruit in water. MJ has a pleasant aroma and its chemical properties result in surface binding to polymeric materials, which may prolong the presence of MJ in storage rooms or fumigation chambers. JA, being more soluble in water, is suitable for use in solution as a dip. When applied at low concentrations, jasmonates are potential post harvest treatments to enhance natural resistance and to reduce decay in fruit. Since they are naturally occurring compounds and are given in low doses, jasmonates may provide a more environment-friendly means of reducing the current chemical usage.

Glucosinolates

Among natural substances with potential antimicrobial activity are the glucosinolates, a large class of approximately 100 compounds produced by members of the family Crucifereae, with well-documented activity (Fenwick *et al.* 1983). Hydrolysis of glucosinolates produces D-glucose, sulphate ion and a series of compounds such as isothiocyanate (ITC), thiocyanate and nitril. The antifungal activity of six glucosinolates has been tested on several post harvest pathogens viz *B. cinerea*, *Rhizopus stolonifer*, *Monilinia laxa*, *Mucor piriformis* and *P. expansum*, both *in vitro* (Mari *et al.* 1993) and *in vivo* (Mari *et al.* 1996c). Of the six ITCs tested, the ITC from glucoraphenin showed the highest effectiveness after 6 days at 20°C, against *M. laxa*, *B. cinerea* and *M. piriformis*. The effectiveness of the ITC from glucoraphenin against *M. laxa* was assayed in two further trials to test the effect of ITC concentration on different concentrations of inoculum and to determine the duration of the curative effect of this ITC. The ITC concentration directly affected fungus control capacity. The highest ITC concentration (3.6 mg/ml) afforded pathogen control at the highest level of pathogen concentration (10^6 conidia/ml) after 6 days at 20°C. Its curative effect was evident up to 40 h after inoculation. Allyl-isothiocyanate (AITC), a naturally occurring flavour compound in mustard and horseradish, has a well-documented antimicrobial activity (Ishiki *et al.* 1992; Delaquis and Mazza 1995). This volatile substance can be employed successfully as a gaseous treatment before storage. Exposure of pear fruit to an AITC-enriched atmosphere resulted in good control of blue mould, including a TBZ-resistant strain on pears (Mari *et al.* 2002). The use of AITC, produced from purified sinigrin or from *Brassica juncea*, against *P. expansum* appears very promising as an economically viable (100 ml of AITC costs about US$25, Merck India Ltd.) alternative with moderately low impact on the environment (Mari *et al.* 2003).

Essential oils

Natural pesticides based on plant essential oils (EOs) could represent alternative crop protectants. The EOs produced by different plant species are in many cases biologically active and have antimicrobial, allelopathic, antioxidant and bioregulatory properties (Vaughan and Spencer 1991; Caccioni and Guizzardi 1994). The essential oils are complex mixtures of natural substances viz. terpenes, sesquiterpenes, aldehydes, ketones and phenolics produced by plants. These EOs are thought to play a role in plant defense mechanisms against phytopathogenic microorganisms (Mihaliak *et al.* 1991). The oils, such as lemon, orange, mustard, and anise give fruits and seeds their characteristic odor and taste. Many are found in common foods, and many are approved as food flavorings by FDA. A wide variety of EOs and their constituents possess varying degrees of antimicrobial properties. The EOs are derived from various parts of the plant, such as flowers, fruits, leaves, and wood. Sometimes the chemicals in the oil, as well as the oil itself, are registered as pesticide active ingredients. It is also fairly common for two or more oils to be used in the same commercial product.

The antimicrobial effects of EOs or their constituents on post harvest pathogens have been quite extensively studied (Bishop and Thornton 1997). The advantage of essential oils is their bioactivity in the vapour phase, a characteristic that makes them attractive as possible fumigants for stored product protection. Control of the storage pathogen *B. cinerea* on Dutch white cabbage (*Brassica oleracea* var. *capitata)* by the EOs of *Melaleuca alternifolia* in *in vitro* conditions has been investigated (Bishop and Reagon 1998). Effect of *Cymbopogon nardus* EO on growth and morphogenesis of *Aspergillus niger* has been tested (Bellerbeck *et al.* 2001). The antifungal activity of EO of *Culamintha sylvatica* has also been tested (Hidalgo *et al.* 2002). However, the *in vivo* efficacy and practical activity of only a few of the EOs have been studied. There are also some reports on enhancing storage life of fruits and vegetables by controlling their fungal rot. The potential of using EOs by spraying or dipping to control post harvest decay has been examined in fruits viz. cherries, citrus fruits, apple, peaches and cabbage (Tiwari *et al.* 1988; Smid *et al.* 1994; Dixit *et al.* 1995). Thymol is an EO component from thyme (*Thymus capitatus*) and has been used as medicinal drug, food preservative, and beverage ingredient (Jain 1985; Mansour *et al.* 1986). Fumigation of sweet cherries with thymol was effective in controlling post harvest grey mold rot caused by *B. cinerea* (Chu *et al.* 1999), and brown rot caused by *M. fructicola* (Chu *et al.* 2001). Fumigation with thymol at 30 mg/l reduced the incidence of grey mold rot from 35% to 0.5% 100 g of thymol costs US$12 (Loba Chmie, India) and it can be assumed that one liter of its solution (30 mg/l) will be effective in treating more than 1000 cherries at a time by the method of fumigation or spray. Its lower price indicates the probable cost effective application. Liu *et al.* (2002) also found that thymol was more effective for controlling brown rot symptoms on apricots, and fumigation of plums with relatively low concentrations such as 2 or 4 mg/l can greatly reduce post harvest decay without causing any phytotoxicity. The US Food and Drug Administration lists thymol, thymol EO and thyme (spice) as food for human consumption, as well as food additives (www.epa.gov/oppsrrd1Reds/factssheets/3143facts). Thymol was initially registered as a pesticide in US in 1964.

Shelf life and safety of some perishable foods by EOs have been remarkably improved (Ponce *et al.* 2004; Holley and Patel 2005). The EO of *Salvia officinalis* has also shown practical potency in enhancing the storage life of some vegetables by protecting them from fungal rot (Bang 1995). Treatment of oranges by fumigation with the EOs of *Mentha arvensis* (100 µl/l), *Ocimum canum* (200 µl/l) and *Zingiber officinale* (200 µl/l) has been found to control blue mold, thereby enhancing shelf life (Tripathi 2004). Plaza *et al.* (2004) evaluated the potential of thyme, oregano, clove and cinnamon EOs against *P. digitatum* and *P. italicum* on citrus fruits. The post harvest quality of strawberry and tomato fruit was evaluated after treatment with eucalyptus and cinnamon volatile EO vapours (Tzortzakis 2007). However, the variations in the fungicidal action of the compounds seem to depend on solubility as well as on the capacity to interact with cytoplasmic membrane.

Although the fungitoxic properties of the volatile constituents of higher plants have been reported, little attention has been paid to the fungitoxicity of these substances in combination. This information is desirable since the fungitoxic potency of most of the fungicides has been reported to be enhanced when combined (Levy *et al.* 1986; Gullino and Garibaldi 1987; Migheli *et al.* 1988; Pandey and Dubey 1997). The enhancement of fungitoxic potential of mixtures of the EOs may be due to the joint action of two or more substances present in the oils (Scardavi 1966). This synergism would be beneficial in post harvest protection because the pathogen would not easily produce resistance against the components.

It is important to remember that just because a pesticide is derived from a plant does not mean that it is safe for humans and other mammals or that it cannot kill a wide variety of other life. However, some botanical pesticides can be quite toxic to humans and should not be used on plants for human consumption. For example methyl salicylate (oil of wintergreen) is commonly used as a food flavoring, but it can be quite toxic in large doses (Jonathan and Davis 2007).

Plant extracts

Plant extracts for the control of plant diseases are emerging as alternatives to conventional fungicides as they are generally safe to humans and environmentally friendly. The preservative nature of some plant extracts has been known form centuries and there has been renewed interest in the antimicrobial properties of extract from aromatic plants. Some plants extracted in different organic solvents have shown inhibitory action against different storage fungi (Singh *et al.* 1993; Hiremath *et al.* 1996; Rana *et al.* 1999; Okigbo and Pandalai 2005).

The phytochemical investigations of most plants have resulted in the isolation of active principles. Theses compounds when tested against post harvest fungi have shown pronounced antifungal activity. Four compounds, irilin A, irilin B, the flavonone dihydrowogonin and sesquiterpene pygmol, were isolated from dichloromethane extract of the aerial parts of *Chenopodium procerum*. The latter three compounds inhibited the growth of the plant pathogenic fungus *Cladosporium cucumerinum* (Bergeron *et al*. 1995). A naturally occurring compound isolated from the flavedo tissue of 'Star Ruby' grapefruit (*Citrus paradise*) 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). *In vitro* inhibition of *Botryodiplodia theobromae* causing Java black rot in sweet potato was induced by phenolic compounds, chlorogenic acid giving the highest *in vitro* inhibition followed by pyrogallol, pyrocatechol, phenol and resorcinol. Low concentrations of phenols are required by the fungus during normal metabolism but higher concentrations are inhibitory to growth (Mohapatra *et al*. 2000). The phytochemical investigation of a methanolic extract of *A. nilotica* resulted in isolation of Kaempferol. Kaempferol has shown antifungal activity against *P. italiccum* at 500 µg/l (Tripathi *et al*. 2002). The aqueous extract of *Acacia nilotica* showed pronounced antifungal activity against *P. italicum* and enhanced the shelf life of orange fruits. The petroleum ether and ethanolic extract of *Origanum syriacum*, *Centaurea pallescens*, *Cichorium intybus*, *Eryngium creticum*, *Salvia fruticosa*, *Melia azedarach*, *Foeniculum vulgare*, *Inula viscosa* and *Cichorium intybus*, were tested against fungi *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. melonis, and *Verticillium dahlia*. Wild marjoram (*Origanum syriacum*) PE extract showed the highest and widest range of activity. It resulted in complete inhibition of mycelial growth of six of eight fungi tested and also gave nearly complete inhibition of spore germination of the six fungi included in the assay (Abou-Jawdah *et al*. 2002). Antifungal activities of extracts of sixteen plants were tested against *Ceratocystis paradoxa* which causes soft rot of pineapples. *Xanthium strumarium* was the most effective followed by *Allium sativum*. The effectiveness of various extracts against *C. paradoxa* was in the decreasing order of *Meriandra bengalensis*, *Mentha piperita*, *Curcuma longa*, *Phlogacanthus thyrsiflorus*, *Toona ciliata*, *Vitex negundo*, *Azadirachta indica*, *Eupatorium birmanicum*, *Ocimum sanctum* and *Leucas aspera*. Treatment of pineapple fruits infested with *C. paradoxa* by *X. strumarium* extract reduced the severity of the disease (Damayanti *et al*. 1996). During another study antifungal activity and minimal fungicidal concentration (MFC) of extracts of garlic, bakeri garlic, Chinese leek, Chinese chive, scallion, onion bulb and shallot bulb against *Aspergillus niger*, *A. flavus* and *A. fumigatus* were examined. These *Allium* plants possessed antifungal activity, with garlic showing the lowest MFC. With the exception of scallion, the inhibitory effect of *Allium* plants against three *Aspergillus* species decreased with increasing incubation and heating temperature . Acetic acid treatments of the extracts increased the inhibitory effect for all plants against the tested fungi (Yin and Tsao 1999). The fungistatic activity of six aqueous extracts of plants were tested against *Aspergillus candidus*, *Aspergillus niger*, *Penicillium* sp. and *Fusarium culmorum*. The plants were *Anthemis nobilis* L., *Cinnamomum verum* J. Presl., *Lavandula stoechas* L., *Allium sativum* L., *Malva sylvestris* L. and *Mentha piperita* L. The more concentrated extracts of *A. nobilis* and *M. sylvestris* inhibited totally the growth of the tested fungi with *M. sylvestris* the most effective one (Magro *et al*. 2006). The inhibitory effect of water-soluble extracts of garlic bulbs, green garlic, green onions, hot peppers, ginger, Chinese parsley, and basil on the growth of *Aspergillus niger* and *Aspergillus flavus* was examined. Garlic bulbs, green garlic, and green onions showed an inhibitory effect against these two fungi (Yin and Cheng 1998). Investigation on the mode of action and practical activity is required so as to recommend their formulation in control of post harvest diseases.

Propolis

Propolis is a natural resinous substance obtained from leaf bud and bark of *Poplar* spp. and leaf resin of *Baccharis dracunuculifolia* and *Clusia rosea*. The biological role of propolis in trees is to seal wounds and defend against bacteria, fungi and insects. Propolis contains protein, amino acids, vitamins, minerals and flavonoids (Moreira 1986; Stangaciu 1998; Walker and Crane 1987). 'Typical' propolis has approximately 50 constituents, primarily resins and vegetable balsams (50%), waxes (30%) and essential oils (and pollen (5%) (Katircioglu and Mercan 2006). However, the chemical composition of propolis is quite complicated. To date there is no exact data about the chemical composition of propolis. The composition of proteins amino acids, vitamins, minerals and flavonoids vary depending upon the plant species and perhaps its plant origin make them so variable in their composition. Its compounds and biological activities depend on many different factors such as the geographical regions, collection time and plant source (Sforcin *et al* 2000; Bankova *et al*. 2002; Bankova 2005). It has antibiotic activity, antibacterial and antifungal activity (Tosi *et al*. 1996). Propolis has been found to inhibit the post harvest pathogens *B. cinerea* and *P. expansum* (Lima *et al*. 1998).

ANTIMICROBIAL SUBSTANCES FROM SOIL

Fusapyrone and deoxyfusapyrone

An antifungal metabolite named fusapyrone has been purified and characterized from soil. Source of fusapyrone in soil is basically from fungi *Fusarium*. Fusapyrone and deoxyfusapyrone, 2, α-pyrone originally isolated from rice culture of *Fusarium semitectum* were tested in several biological assays and showed considerable antifungal activity against several plant pathogenic fungi. The inhibitory activity of fusapyrone against the growth of *B. cinerea* has been assayed *in vitro* and *in vivo* on grapes. Significant inhibition of conidia germination of *B. cinerea* has been recorded and grapes treated with 100 µg/ml of fusapyrone inhibited the development of grey mold on damaged grapes (Altomare *et al*. 1998). Low toxicity towards animals and absence of phytotoxic effects of fusapyrone have promoted its use in control of *B. cinerea* on grapes and other crops (Altomare *et al*. 2000).

NATURAL ANIMAL-BASED ANTIMICROBIAL SUBSTANCES

Chitosan

Chitosan a given name to a deacetylated form of chitin as a natural biodegradable compound derived from crustaceous shells as crabs and shrimps, whose main attribute corresponds to its polycationic nature. Chitosan is a soluble form of chitin. Chitosan and its derivatives have plant protective and antifungal properties. Chitosan has been proven to control numerous pre- and post harvest diseases on various horticultural commodities. It has been reported that both soil and foliar plant pathogens fungal, bacterial and viral may be controlled by chitosan application. They can trigger defensive mechanisms in plants against pathogenic attacks at very low concentrations. They can also be used in solution, powder form or as wettable coatings of seeds and fruits (Choi *et al*. 2002). Chitosan in aqueous solution at concentrations of 0.25, 0.5, 1.0 and 2.0% (w/v) has been used as an alternative control agent against blue mould (*Penicillium expansum*) in harvested 'Red Delicious' apple fruit and greater reductions of the disease was observed for concentrations of 1 and 2% and for inoculation times 48 and 96 h. It has been shown that chitosan induces resistance in the fruit rather than merely inhibiting the pathogen directly (Capdville *et al*. 2002).

According to El Ghaouth *et al*. (1992a) microscopical observations indicate that chitosan has a direct effect on the morphology of the microorganisms reflecting its fungicidal or fungistatic potential. Chitosan at a concentration greater than 1.5 mg/ml induced morphological changes in *R. stolonifer*. Mechanisms by which chitosan coatings reduced the decay of strawberries appear to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase and β-1,3-glucanase. In addition to its microbial activity other studies strongly suggest that chitosan induces a series of defense reactions correlated with enzymatic activities (Mauch *et al*. 1984). Chitosan has been shown to increase the production of glucanohydrolases, phenolic compounds and synthesis of specific phytoalexins with antifungal activity, and also reduces macerating enzymes such as polygalcturonases, pectin methyesterase, etc. (Doares *et al*. 1995). In addition, chitosan induces structural barriers for example it induces the synthesis of a lignin-like material. For some horticultural and ornamental commodities, chitosan increased harvested yield. Due to its ability to form a semi-permeable coating, chitosan extends the shelf life of treated fruit and vegetables by minimizing the rate of respiration and reducing water loss (El Ghaouth *et al* 1992a, 1992b). As a nontoxic biodegradable material, as well as an elicitor, chitosan has the potential to become a new class of plant protectant, assisting towards the goal of sustainable agriculture.

CONCLUSION

Currently, several promising biological approaches that include microbial antagonists, naturally occurring antifungal compounds have been advancing as potential alternatives to synthetic fungicides for post harvest disease control. However, there are some limitations of using microbial antagonists viz. limited spectrum of activity and efficacy under some environmental conditions; specificity against various diseases or fruits; and lack of eradicative activity. Therefore it would be inappropriate to equate biological control with chemical treatment without considering the advantages and limitations of both the methods. Unfortunately, the efficacy of some biological control antagonists evaluated under simulated and actual commercial conditions has been irregular unless they are combined with other treatments, such as low rates of fungicides. More recently, natural plant extracts or products have become extremely popular for controlling plant diseases Encouraging results on the use of natural products to control post harvest rotting indicate the potential for development of natural pesticides that would be as effective as synthetic fungicides, and presumably safer for man and the environment. Although the exploitation of natural products to protect the post harvest decay of perishable products is in its infancy, these products have the potential to be safe fungicides and may replace synthetic ones. The product for post harvest disease management should be effective even for short duration treatments due to the limited post harvest life of perishables. Several studies deal with products such as salicylic acid essential oils, acetaldehyde and ethanol that have the potential to be used in the post harvest environment (Barkai-Galon 2001; Korsten 2004) but the studies of Obagwa and Korsten (2003) for instance, describe the some off odour effect of these natural products which will prevent their commercialization. Therefore the treatment should not have an effect on quality parameters such as acidity, flavour and aroma (Nagy and Shaw 1990; Oberhofer *et al*. 1999). The lowest suitable dose of the chemicals for practical application should also be determined. Keeping in view the merits of the botanicals as post harvest fungitoxicants, the products which are found efficacious during *in vitro* testing, should be properly tested for their practical potency based on *in vivo* trials, organoleptic tests and a safety limit profile (de Roever 1999; Schilter *et al*. 2003).

It is without a doubt that a more sophisticated holistic approach to total product management will ensure quality and safety and provide retailers with the desired extended shelf life. Integrating various methods may provide a more durable, consistent, sustainable and practical solution to eliminate pathogens. Now we have a number of alternative disease control options and each option has some potential. However, most of the work done to date has not been successfully adopted on a commercial scale. Under these situations we must expect that the slow uptake of new technologies will change and start to accelerate and to solve the dilemma of post harvest disease control of perishables.

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