

Review

Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities

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Abstract

Chitosan, a given name to a deacetylated form of chitin, is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes corresponds to its polycationic nature. Chitosan has been proven to control numerous pre and postharvest 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. Microscopical observations indicate that chitosan has a direct effect on the morphology of the chitosan-treated microorganism reflecting its fungistatic or fungicidal potential. In addition to its direct microbial activity, other studies strongly suggest that chitosan induces a series of defence reactions correlated with enzymatic activities. 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 polygalacturonases, pectin metil esterase etc. In addition, chitosan induces structural barriers for example inducing the synthesis of lignin-like material. For some horticultural and ornamental commodities, chitosan increased harvested yield. Due to its ability to form a semipermeable coating, chitosan extends the shelf life of treated fruit and vegetables by minimizing the rate of respiration and reducing water loss. 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.

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1. Introduction

During the last decade, control of diseases of horticultural commodities has become increasingly difficult. In spite of the great advantages they have brought to agriculture development, the excessive use of fungicides has taken its toll environmentally and on human health (Carson, 1962). There is a great demand for residue-free fresh produce. Additionally, re-registration procedures of broad-spectrum fungicides and the increasing resistance of fungal strains to fungicides are some of the main problems that face growers (De Waard et al., 1993; Bruton, 1994).

There is a worldwide trend to explore new alternatives that control postharvest pathogenic diseases, giving priority to methods that reduce disease incidence and avoid negative and side effects on human health as a result of the excessive application of synthetic fungicides. In addition, the emergence of fungicide-resistant strains of microorganisms and the continuous rigorous regulation of fungicide use and disposal has reduced the possibility to conceive control strategies based on chemicals (Johnson and Sangchote, 1994).

The biodegradable nature of natural compounds derived from animal and plants have interested plant pathologists. Among them chitosan, a high molecular polymer, nontoxic, bioactive agent has become a useful appreciated compound due to its fungicidal effects and elicitation of defence mechanisms in plant tissues (Wilson et al., 1994; Terry and Joyce, 2004).

2. Definition and application

Chitosan, deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps (Sandford and Hutchings, 1987; Sandford, 1989). Chitin and chitosan are polysaccharides, chemically similar to cellulose differing only by the presence or absence of nitrogen. Chitosan is a low acetyl form of chitin mainly composed of glucosamine, 2-amino-2-deoxy- β -D-glucose (Freepons, 1991). The positive charge

of chitosan confers to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as cosmetology (lotions, hair additives, facial and body creams) (Lang and Clausen, 1989), food (coating, preservative, antioxidant, antimicrobial) (Sapers, 1992; Pennisi, 1992; Fang et al., 1994; Roller and Covill, 1999; Benjakul et al., 2000; Shahidi et al., 2001), biotechnology (chelator, emulsifier, flocculent) (Hirano, 1989; Sandford, 1989) pharmacology and medicine (fibers, fabrics, drugs, membranes, artificial organs) (Muzarelli, 1989; Kulpinsky et al., 1997; Nishimura, 1997; Liu et al., 2001) and agriculture (soil modifier, films, fungicide, elicitor) (Hoagland and Parris, 1996; Lafontaine and Benhamou, 1996; Makino and Hirata, 1997; Ren et al., 2001).

3. Fungicidal activity of chitosan

The fungicidal activity of chitosan has been well documented both in in vitro and in situ studies. Literature generally reports that the level of inhibition of fungi is highly correlated with chitosan concentration, indicating that chitosan performance is related to the application of an appropriate rate. It is believed that the polycationic nature of this compound is the key to its antifungal properties and that the length of the polymer chain enhances its antifungal activity (Hirano and Nagao, 1989). An additional explanation includes the possible effect that chitosan might have on the synthesis of certain fungal enzymes (El Ghaouth et al., 1992d).

Recent studies have shown that chitosan is not only effective in halting the growth of the pathogen, but also induces marked morphological changes, structural alterations and molecular disorganization of the fungal cells (Benhamou, 1996; El Ghaouth et al., 1999; Ait Barka et al., 2004).

3.1. Effect of chitosan on in vitro fungal development

There is strong evidence that mycelial growth can be inhibited or retarded when the growth media of fungi

are amended with chitosan. For example, as chitosan concentration increased ($0.75\text{--}6.0\text{ mg ml}^{-1}$), the radial growth of *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, decreased (El Ghaouth et al., 1992c). The same effect was reported on *Sclerotinia sclerotiorum* when chitosan concentrations increased from 1% to 4% (Cheah et al., 1997). Other studies showed a linear decrease of growth of *Rhizoctonia solani* as the chitosan concentration gradually increased from 0.5 to 6.0 mg ml^{-1} (Wade and Lamondia, 1994). Mycelial growth of *Fusarium solani* f. sp. *phaseoli* and *F. solani* f. sp. *pisi* was inhibited at the minimum concentrations of 12 and $18\text{ }\mu\text{g ml}^{-1}$, respectively (Hadwiger and Beckman, 1980; Kendra and Hadwiger, 1984). Other studies reported a complete growth inhibition of fungi such as *F. oxysporum*, *R. stolonifer*, *Penicillium digitatum* and *C. gloeosporioides* at concentrations of 3% (Bautista-Baños et al., 2003, 2004b). *B. cinerea*'s radial growth and mycelium dry mass was lower after 5 d of incubation on nutrient agar amended with 5% and 10% chitogel (a formulated chitosan solution), respectively (Ait Barka et al., 2004). The long-term fungicidal effect of chitosan can also be related to concentration and incubation time. For example inhibition of *F. oxysporum* f. sp. *radicis-lycopersici* grown at two of the lowest concentrations (1.0 and 2.0 mg ml^{-1}) decreased with increased incubation time (Benhamou, 1992) while for *A. niger* chitosan efficacy was highly dependant on incubation time (168 h) (Plascencia-Jatomea et al., 2003). The effect varies with fungal species. For example, growth of *R. nigricans* was not affected by chitosan, contrary to the inhibition observed on *R. stolonifer* when grown on this compound (Allan and Hadwiger 1979; El Ghaouth, 1992c; Bautista-Baños et al., 2004b). Overall, sporulation of fungi treated with chitosan is generally reported to be lower than in untreated fungi. Moreover, in some reports no spores were observed after chitosan treatment. The inhibition of spore formation is supported by the effect on *F. oxysporum*, *R. stolonifer*, *C. gloeosporioides*, *A. alternata* f. sp. *lycopersici* and *A. niger* (Bhaskara Reddy et al., 1998; Bautista-Baños et al., 2003, 2004b; Plascencia-Jatomea et al., 2003). Nevertheless, chitosan sometimes stimulates sporulation. Spore formation of *P. digitatum* when grown on chitosan was significantly greater than the control treatment at both concentrations of chitosan tested (0.5% and 1.5%). A similar result was reported on *A. alternata* f. sp. *lycopersici* grown at sub-lethal doses of chitosan ($100\text{--}500\text{ }\mu\text{g ml}^{-1}$) (Bhaskara Reddy et al., 1998; Bautista-Baños et al., 2004b). Those authors indicate that this high sporulation might have been due to a stress response induced by this polymer. Spore viability can be affected by chitosan. In one study a concentration of 0.75 mg ml^{-1} upwards reduced spore viability and germ tube growth of both *B. cinerea*

and *R. stolonifer* (El Ghaouth et al., 1992a), while in other experiments it was shown that concentrations from 1.5 to $100\text{ }\mu\text{g g}^{-1}$ markedly reduced *B. cinerea* spore viability (Ben-Shalom et al., 2003). The long-term effect of chitosan on spore viability has also been demonstrated, for example, by the low percentage germination of uredospores of *Puccinia arachidis* previously treated with various chitosan concentrations from 100 to $1000\text{ }\mu\text{g g}^{-1}$ after continuous washes with distilled water (Sathiyabama and Balasubramanian, 1998).

The mechanism by which chitosan affects the growth of several phytopathogenic fungi has not been fully elucidated, but several hypotheses have been postulated. Because of its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (Leuba and Stossel, 1986). Other mechanisms mentioned in the literature are the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis (Hadwiger et al., 1986) and the chelation of metals, spore elements and essential nutrients (Cuero et al., 1991).

3.2. Changes of fungal morphology due to the effect of chitosan

Microscopic observation of fungi treated with chitosan revealed that it can affect the morphology of the hyphae. In experiments with *Trichoderma longibrachiatum* the extreme cell wall of the tip of the hyphae was thinned in presence of chitinase and β -1, 3-glucanases (Arlorio et al., 1992). Other observations carried out on fungi such as *F. oxysporum* f. sp. *radicis-lycopersici*, *R. stolonifer* and *S. sclerotiorum* treated with chitosan showed excessive mycelial branching, abnormal shapes, swelling, and hyphae size reduction (Benhamou, 1992; El Ghaouth et al., 1992a,c; Cheah et al., 1997). Similarly, chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea* and *F. oxysporum* f. sp. *albedinis* (Ait Barka et al., 2004; El Hassni et al., 2004). Ultrastructural studies have also confirmed alterations to hyphae of *R. stolonifer* by chitosan. In that study, swellings and hyphae convolutions, surrounded by loosened cell walls were observed. The integrity of the cell wall of *Rhizopus* becomes markedly altered. Incubation with the wheat germ agglutinin/ovomucoid-gold complex, showed wall portions more intensely labelled. Nevertheless, there was no correlation between cell wall abnormalities originating by contact with chitosan and cellular leakage. In further studies, image analysis was used to measure the effect of chitosan on individual morphological parameters of spores of *C. gloeosporioides*, *R. stolonifer*, *P. digitatum* and

F. oxysporum. Area, length and form of conidia of each of the fungi tested were affected according to fungal species and time of incubation in chitosan solutions (Bautista-Baños et al., 2003, 2004b). Another study reported that spore morphology of *A. niger* was also affected when treated with chitosan (Plascencia-Jatomea et al., 2003).

4. Chitosan as an elicitor of response mechanisms in plants

4.1. Biochemical defence response in preharvest studies

In general, induced defence reactions in plants are highly correlated with enzymatic responses. Several studies have demonstrated that chitosan is an exogenous elicitor of host defence responses, including accumulation of chitinases, β -1,3-glucanases and phenolic compounds, induction of lignification, synthesis of phytoalexins by the infected host tissue and inhibition of host tissue maceration enzymes (Tejchgraber et al., 1991; Arlorio et al., 1992; Fajardo, et al., 1995; Bhaskara Reddy et al., 1997, 1999; Zhang and Quantick, 1998). It has also been reported that chitosan alone increased the amounts of genistein and 2'-hydroxygenistein monopenyls in roots of white lupin and isoflavonoids in the exudates. However, in that study no microorganisms were involved (Gagnon and Ibrahim, 1997).

Studies conducted on the germination process and on chitinase activity of soybean seeds subjected to chitosan glutamate solutions at different concentrations (0.1%, 0.5% and 1.0%) and soaking periods (15 min and 6 h) indicated that the period of exposure to chitosan was more decisive for the increase of chitinase activity in soybean seeds than chitosan concentration (Tejchgraber et al., 1991). In tomato plants, the production of phenolics, phytoalexins or related compounds, induced by chitosan, precedes or coincides with the action of hydrolytic enzymes of *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou and Thériault, 1992). Other studies with peanut seeds confirmed that chitosan enhances the production of preformed free and bound phenolic acids in viable seed tissues (Fajardo et al., 1995). Further studies indicated that chitosan triggers either the de-novo synthesis of phenolic compounds as the first defensive line designed to inhibit growth of this fungus and that the β -1,3-glucans act as a second mechanical barrier for blocking potential invasion by fungal cells and protecting the tissue against phytotoxic substances (Benhamou et al., 1994; Lafontaine and Benhamou, 1996). The same authors pointed out that contact with the pathogen is essential for signalling the plant to mobilize its defence strategy and that plants treated with chitosan were able to express these

defence reactions faster and in a greater degree than the pathogen alone after infection. However, in cucumber plants the induction of the defence response without the antifungal activity of chitosan was not enough to reduce gray mould disease (Ben-Shalom et al., 2003). Antifungal hydrolases were reported on roots and leaves of hydroponically grown cucumber plants treated with chitosan and artificially inoculated with *Pythium aphanidermatum* (El Ghaouth et al., 1994a). Recent reports have shown that chitosan has the capacity to induce resistance to *F. oxysporum* in susceptible tomato plants when applied as a root dressing, foliar spray, and seed dressing by restricting pathogen growth to the outer root tissues and eliciting a number of defence reactions, including structural barriers (Benhamou et al., 1998). This effect may be due to the massive accumulation of fungitoxic compounds at sites of attempted pathogen penetration. Because of its filmogenic property, chitosan may also act as a barrier to the outward flux of nutrients and, consequently, may reduce the availability of nutrients to a level that will not sustain growth of the pathogen. This contention is supported by the fact that fungal cells exposed to chitosan often display signs of nutrient deprivation (El Ghaouth et al., 2000; Ait Barka et al., 2004).

Another example of an induced resistance response was reported in groundnut-treated chitosan, where a significant increase of endogenous salicylic acid, intercellular chitinase and glucanase activities were evidenced. (Sathiyabama and Balasubramanian, 1998).

4.2. Biochemical defence response in postharvest studies

Chitosan was effective in reducing the production of polygalacturonases produced by *B. cinerea* in bell pepper tissues and markedly reduced the maceration of the host cell wall components, pectin and cellulose (El Ghaouth et al., 1997). In studies on fresh strawberries and raspberries with a chitosan coating, there was a significant increase of chitinase and β -1,3-glucanase activities of the fruits as compared with the uncoated controls (Zhang and Quantick, 1998). It was also observed that chitosan partially inhibited the increase in peroxidase activity, associated with tissue browning (Zhang and Quantick, 1997). For tomato, chitosan impaired the production of fungal virulence factors such as cell wall degrading enzymes (polygalacturonase, pectate lyase and cellulose), organic acids (oxalic and fumaric acids), and host specific toxins (alternariol and alternariol monomethylether) and induced production of rishitin (Bhaskara Reddy et al., 1998, 2000b). In table grapes, chitosan enhanced phenylalanine ammonia-lyase activity (the key enzyme of the phenylpropanoid) (Romanazzi et al., 2002). Other enzymatic activities such as peroxidase and polyphenol oxidase activity were elicited in palm roots injected with chitosan (El Hassni

et al., 2004). In the same study, after treatment with chitosan the presence of caffeoylshimick acids (sinapic, *p*-coumaric and ferulic derivatives), reported to be the major phenolic constitutive compounds in date palm roots and known to have antifungal activity and the precursors of lignin, were reported.

4.3. Structural defence response

The role of the elicitation of several defence-related enzymes has also been studied (Bohland et al., 1997; Vander et al., 1998). These enzymes are known to participate in early defence mechanisms and to prevent pathogen infections. Chitosan and chitin oligomers have also been reported to stimulate other systems involved in resistance, such as lipoxygenase and phenylalanine ammonia lyase activities, and lignin formation in wheat leaves (Bohland et al., 1997; Vander et al., 1998).

The induction of structural barriers at sites of attempted fungal penetration is one of the most common processes that occur in response to pathogen invasion. Cellular suberization and lignification among others are elicited during the infection process in some plant organs. Chitosan is reported to restrict, to some extent, fungal penetration and induce the formation of different structural barriers.

Moderate lignification as a result of chitosan treatment and inoculation with *B. cinerea* cell walls was reported in leaves after 48 and 72 h (Pearce and Ride, 1982). Examination with transmission electron microscopy showed evidence of the formation of particular structures and new material. For example, the main host reactions observed on the host cells in tomato roots and leaves which were chitosan-treated and infected by *F. oxysporum* f. sp. *radicis-lycopersici* were: (1) occlusion of xylem vessels by an opaque or fibrillo-granular material or by the formation of a bubble-like structure, (2) coating of secondary thickenings and pit membrane and (3) papillae formation (wall appositions) into the cortex and the endodermis tissues (Benhamou and Thériault, 1992; Lafontaine and Benhamou, 1996). Other host reactions on chitosan-treated roots were contorted epidermal cells (Benhamou et al., 1994). For bell pepper fruit, structural defence responses were observed only in the first tissue layers beneath the ruptured cells such as thickening of the host cell wall, formation of hemispherical and spherical protuberances along the cell walls, and occlusion of intercellular spaces with fibrillar material (El Ghaouth et al., 1994b, 1997). Further studies demonstrated that the combination of two methods of control; chitosan application and biological control with *Bacillus pumilus* increased the host defence reaction of the treated roots (Benhamou et al., 1998). For cucumber plants grown in the presence of nutrient solutions amended with chitosan, and inoculated by *P. aphanidermatum*, the host reactions were similar to those

observed on chitosan-treated tomato roots such as plugging of intercellular spaces with electron-opaque and fibrillar material and papillae formation alongside the host cell wall (El Ghaouth et al., 1994b).

5. Effect of chitosan on pre and postharvest disease

5.1. Control of bacterial and viral diseases

To date, scarce studies have reported bactericidal or bacteriostatic effects of chitosan on plant diseases. In our laboratories the efficacy of chitosan at various concentrations in inhibiting growth of various strains of *Erwinia amylovora* and *Agrobacterium tumefaciens* showed chitosan concentration had a direct effect on bacterial inhibition (unpublished). In studies carried out on foodborne pathogens, there was evidence that bacteria were affected at membrane level disrupting it and increasing cellular leakage (Helander et al., 2001).

The inhibitory effect of chitosan solutions has also been reported on plant diseases caused by viruses and viroids (Pospiezny and Atabekov, 1989; Pospiezny et al., 1991; Pospiezny, 1997). For example, on bean leaves, local infections produced by alfalfa mosaic virus (ALMV) were completely controlled with the highest chitosan concentration (0.1%) either sprayed or added to the inoculum (Pospiezny et al., 1991). Similar inhibition was reported on tomato leaves treated with chitosan at the same concentration and inoculated with potato spindle tuber viroid (Pospiezny, 1997). In these studies systemic resistance was induced by chitosan on various host virus combinations. In general, it was observed that previous chitosan treatments significantly reduced virus infection in various plants.

5.2. Control of preharvest fungal diseases

The potential of chitosan to delay symptoms and to suppress root rots and plant diseases of various horticultural commodities has been confirmed in various investigations. Seed-borne diseases caused by *F. oxysporum* f. sp. *radicis-lycopersici*, and *P. aphanidermatum*, were significantly reduced when tomato seedlings and seeds were dipped in chitosan solutions (Benhamou and Thériault, 1992; Benhamou et al., 1994; El Ghaouth et al., 1994b; Lafontaine and Benhamou, 1996). Chitosan concentrations directly affected the number of root lesions on tomato seedlings after *F. oxysporum* f. sp. *lycopersici* inoculation. As chitosan concentration increased from 0.5 to 2.0 mg ml⁻¹ root infection levels decreased (Benhamou and Thériault, 1992). In further research (Benhamou et al., 1994), it was reported that the combination of chitosan-treated tomato seeds and chitosan-amended soil was more effective in reducing root lesions by this fungus than

when using chitosan separately on seeds or soil. Moreover, with this combination, no symptoms of disease were observed at the end of the treatment. In cucumber plants, no symptoms of root infection were observed when the growing solution was amended with chitosan ($400\ \mu\text{g ml}^{-1}$) (El Ghaouth et al., 1994b). There was better germination and vigour, and lower disease levels caused by *F. graminearum* on chitosan-treated wheat seeds at a range of concentrations from 2 to $8\ \text{mg ml}^{-1}$, than in untreated controls (Bhaskara Reddy et al., 1999). Nevertheless, for strawberry plants, chitosan applications did not show any fungicidal effect. In greenhouse and field experiments inoculated by three strains of *Rhizoctonia fragariae* or by the nematode *Pratylenchus penetrans* there was no suppression of the black root rot complex, in spite of a high chitosan application ($5.0\ \text{mg ml}^{-1}$). An explanation given for this was related to the infection process of *R. fragariae* via cortical cells and the induced response such as lignin deposition only in the vascular tissue compared with other infected chitosan-treated crops (Wade and Lamondia, 1994). The preventive rather than curative effect of chitosan has been proven on groundnut and cucumber plants infected by *P. arachidis* and *B. cinerea*, respectively. For both studies, lower leaf rust and gray mould incidence on leaves were recorded when chitosan was sprayed 24 h or 1 (1000 ppm or 0.1%, respectively) before inoculation (Sathiyabama and Balasubramanian, 1998; Ben-Shalom et al., 2003). An explanation given for these results was associated with the enhancement of salicylic acid and hence enzymatic activity of chitosan-treated groundnut leaves and by the site of binding for *B. cinerea* being occupied by chitosan molecular charges on cucumber plants. In other studies, pearl millet seeds treated with commercial chitosan (Elexa) at different concentrations (1:5, 1:10, 1:15, 1:19 and 1:25) were effective in reducing downy mildew disease caused by *Sclerospora graminicola* under greenhouse and field conditions (Sharathchandra et al., 2004).

5.3. Control of postharvest diseases

Serious market losses of horticultural produce result from postharvest disease development. Numerous reports indicate that chitosan effectively controls postharvest rots during storage, delays the onset of infection and slows down the infection process. In general, the reduction of rots increases with increasing chitosan concentration. In chitosan-treated fruits such as apples, kiwifruit, pears and others significant reduction of storage rots has been recorded (Bautista-Baños et al., 2004c; Du et al., 1997). In strawberries and raspberries chitosan coatings (10 and $15\ \text{mg ml}^{-1}$) reduced two of the main postharvest diseases, gray mould and *Rhizopus* rot. Moreover, chitosan fungicidal performance was equivalent to that of the synthetic fungicides such as

iprodione and thiabendazole (TBZ), commonly used to reduce these diseases (El Ghaouth et al., 1991b, 1992a, b; Zhang and Quantick, 1998). Similarly, on carrots artificially inoculated with *S. sclerotiorum*, and on noninoculated papaya fruit, the performance of iprodione and TBZ, respectively, was lower than chitosan at concentrations of 2% and 4% (Cheah et al., 1997; Luna et al., 2001). Nonetheless, it has been reported that chitosan coatings are not always more effective than synthetic fungicides, as was demonstrated on chitosan-treated litchi which delayed the infection process during the 33 d storage period, but was not as effective as TBZ in controlling rots (Zhang and Quantick, 1997). A similar lower fungicidal effect of chitosan was reported on peaches, artificially inoculated with *Monilinia fructicola* compared to the fungicide prochloraz (Li and Yu, 2000). In other studies carried out with bell pepper treated with chitosan ($10\ \text{mg ml}^{-1}$), gray mould disease symptoms were retarded up to 7 d after storage (El Ghaouth et al., 1997). For various fruits such as sweet cherries, table grapes and oranges, the fungicidal activity of chitosan was effective both on artificially inoculated or uninoculated fruit (Romanazzi et al., 2001).

In inoculated apple fruit, pretreated with an array of alternatives before storage, chitosan at 1% and 2% was effective in reducing *P. expansum* during controlled storage, followed by ultraviolet irradiation treatment and harpin protein (de Capdeville et al., 2002). The possibility of enhancing the fungistatic or fungicidal activity of chitosan by means of a combination of different treatments has been explored as well. For example, sweet cherries treated with different chitosan concentrations (0.1%, 0.5% and 1.0%) and hypobaric treatments (0.50 and 0.25 atm) resulted in better control of postharvest rots than chitosan alone (Romanazzi et al., 2003). The additive or synergistic effect between antagonistic microorganisms, sodium carbonate and glycolchitosan at 0.2% to control *P. expansum* is reported on apples and citrus fruit (El Ghaouth et al., 1999, 2000). Further studies demonstrated that in some fruits, chitosan had a preventive rather than a curative effect. Anthracnose disease of papaya fruit was better controlled when the fruit was dipped in a chitosan solution of 1.5% before rather than after artificially inoculating fruit (Bautista-Baños et al., 2003). Sclerotinia incidence and size rot in carrots were reduced when hydrolysed chitosan (0.2%) was applied 3 d before inoculation (Molloy et al., 2004). Preharvest chitosan applications in field trials have reduced postharvest disease after fruit storage. Sprays of chitosan at various concentrations of 2, 4 and $6\ \text{g l}^{-1}$ during an interval of 10 d before harvest on strawberry plants reduced gray mould during fruit storage (Bhaskara Reddy et al., 2000a). Likewise, postharvest control of this disease was reported on grapes treated in the field with chitosan, regardless of

concentration. Fruit infection index was significantly lower than that of the untreated fruit and similar to those treated with the fungicide procymidone. On fruit previously sprayed with chitosan (0.1%, 0.5% or 1.0%) 21 or 5 d before harvesting (Romanazzi et al., 2002). In our laboratory, we have observed that the degree of polymerization of the chitosan applied, does not have a direct effect on the antifungal potential of chitosan. For example, *Rhizopus* rot development was significantly lower in chitosan-treated tomato (1.0%, 1.5% and 2.0%) than the untreated fruit, regardless of the degree of polymerization of this compound. However, chitosan was less effective than the fungicide dicloran (Bautista-Baños and Bravo-Luna, 2004a).

6. Effect of chitosan on the postharvest quality of various horticultural commodities

An additional positive effect of chitosan coatings is related to its ability to extend the storage life of fruits and vegetables. Chitosan forms a semipermeable film that regulates the gas exchange and reduces transpiration losses and fruit ripening is slowed down. Because chitosan is applied as a coating, generally respiration rate and hence water loss is reduced. This effect has been reported for numerous horticultural commodities such as tomatoes, strawberries, longan, apples, mangoes, bananas, bell peppers, etc. (El Ghaouth et al., 1991a, 1992e; Du et al., 1997, 1998; Jiang and Li, 2001; Kittur et al., 2001). The efficacy of chitosan in reducing production of internal CO₂ is reported on tomatoes, tangerines and pears (El Ghaouth et al., 1992e; Du et al., 1997; Salvador et al., 2003). Chitosan coatings and the storage temperature might be associated with reduced CO₂ production. On cucumbers and bell peppers, respiration rate was lower at 13 °C than at 20 °C (El Ghaouth et al., 1991a). Because an inhibition of CO₂ often results from a chitosan coating, consequently ethylene production of the commodity is also reduced. Both inhibitory effects were reported in peaches and tomatoes coated with chitosan (Li and Yu, 2000; El Ghaouth et al., 1992e). Several examples indicate that the loss of firmness of the chitosan-treated fruit such as strawberries, raspberries, tomatoes, peaches, papayas and others was delayed during the storage period and various reports indicate that the treated fruit was firmer at the end of storage (El Ghaouth et al., 1991b, 1992b, e; Li and Yu, 2000; Bautista-Baños et al., 2003). Chitosan sprays during preharvest life of strawberry fruit, indicated that the chitosan concentration applied, together with storage temperature and time after harvest, resulted in firmer fruit (Bhaskara Reddy et al., 2000a). External appearance of fruits and vegetables is generally improved, for example, the colour of fruit is generally retained when coated with chitosan. However,

on Japanese pears and papaya there was a slight colour development, while a deeper green colour than control fruit was reported on cucumber and bell peppers (El Ghaouth, 1991a, 1992e; Woods et al., 1996; Du et al., 1997; Jiang and Li., 2001; Luna et al., 2001). In general, anthocyanin degradation on chitosan-treated fruit is retarded. This has been demonstrated in litchi, strawberries and raspberries (Li and Chung, 1986; Zhang and Quantick, 1997, 1998). However, another report mentions a contrary effect; a synthesis of anthocyanins on strawberries treated with chitosan (El Ghaouth et al., 1991b). An explanation for these contradictory reports could be associated with cultivar, source of chitosan and inoculum. Generally, at the end of the storage period, titratable acidity was reported to increase on the chitosan-treated commodity (strawberries, tomatoes, and peaches), while in other crops such as mangoes and longan, acidity was slowly reduced, associating this decrease with loss of eating quality (El Ghaouth et al., 1992e; Li and Yu, 2000; Jiang and Li, 2001; Srinivasa et al., 2002). After storage, total solid solubles (TSS) of chitosan-treated fruits differed according to the commodity. Lower TSS than control fruit were reported in mangoes and bananas coated with chitosan while higher values were reported on treated peaches. However, other studies reported that TSS of chitosan-dipped papayas and zucchinis were the same as the untreated fruit (Du et al., 1997; Kittur et al., 2001; Constantino et al., 2001; Srinivasa et al., 2002; Bautista-Baños et al., 2003). The reducing sugar content of fruit is also affected by chitosan coating. Lower reducing sugars than the untreated fruit were reported for bananas at the end of the storage period (Kittur et al., 2001). However, contradictory reports about the levels of reducing sugars of chitosan-treated mangoes were recorded. An explanation for this might be the mode of application of chitosan onto the fruit. In the first study, mango fruit were carton-packed and covered with a chitosan film and the reducing sugars were higher than the control while, in the second study, mango fruit were dipped in a chitosan solution, and these fruit had lower reducing sugars than control fruit (Kittur et al., 2001; Srinivasa et al., 2002), indicating a higher reduction of the fruit metabolism in the dipped than in the nondipped fruit. The content of ascorbic acid was also evaluated in chitosan-treated mango and peaches (Li and Yu, 2000; Srinivasa et al., 2002). In those studies, the content of this vitamin in the treated mango gradually decreased during the storage period and was lower than in untreated fruit. However, for peaches, the content of ascorbic acid was higher in chitosan-treated fruit when compared within untreated or in prochloraz-treated fruit after a 12d-storage period. Although few studies report the effect of chitosan on the sensory attributes of the treated commodity, generally, flavour and taste were reported to be constant. Mango and strawberry

fruit treated with chitosan scored superior sensory attributes compared with untreated fruit during the storage periods of 21 and 15 d, respectively (Li and Chung, 1986; Kittur et al., 2001). For strawberry fruit, the addition of a calcium salt to chitosan showed the best sensory attributes after 21 d-storage period (Li and Chung, 1986; Kittur et al., 2001). In further studies chitosan coatings on strawberry and packed for 7 d had a bitter taste only on day 0 (Devlieghere et al., 2004). Papayas treated with chitosan had less flavour when treated with chitosan, which might be due to the delay of the ripening process (Luna et al., 2001).

7. Effect of chitosan on yield at harvest

There are few published references related to the effect of chitosan on the subsequent yield but these reports indicated that preharvest application of chitosan solutions increased fruit yield at harvest. For example, an increase of tomato yield at harvest was highly correlated with the concentration of chitosan applied to soil inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* before seedling transplanting (Lafontaine and Benhamou, 1996). However, when fungal inoculation was not involved, fruit yield from chitosan-treated soil and untreated was similar, indicating that it is disease reduction by chitosan that is highly correlated with higher fruit yield. Similar results were obtained in experiments with *Eustoma grandiflorum* (Lisianthus) where soil amended with chitosan (1%) accelerated the flowering period and increased mass and number of flowers (Ohta et al., 1999). Apparently, plants showed a significant higher fresh and dry mass and number of leaves in the chitosan-treated soil than the untreated, therefore inducing greater flower production.

8. Conclusions

Chitin is considered as the second most abundant natural polymer and one of the most widely distributed throughout nature (Sandford, 1989). In patent literature, the number of applications of chitin, chitosan and their derivatives has been increasing steadily in the last decade. Chitosan has been shown to be a versatile nontoxic material with a dual effect: it controls pathogenic microorganisms and activates several defence responses inducing and/or inhibiting different biochemical activities during the plant-pathogen interaction. To date, there is enough evidence indicating that after chitosan application, plants can acquire enhanced tolerance to a wide variety of pathogenic microorganisms, indicating that the use of natural elicitors such as chitosan might assist in the goal of sustainable agriculture.

Studies on the commercial application of chitosan in the field to reduce pathogenic microorganisms are scarce. The beneficial effects of chitosan might be extended from the field through to the storage of numerous horticultural commodities. A combination of biological techniques that complement the additive and/or synergistic effects between chitosan and other natural compounds or combined with antagonists i.e. microorganisms might provide alternatives to control plant diseases.

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