Crop Protection 28 (2009) 36–40

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Assessment of chitosan for inhibition of *Colletotrichum* sp. on tomatoes and grapes

Z. Muñoz, A. Moret*, S. Garcés

Departament de Biologia Vegetal, Facultat de Biologia, Avgda. Diagonal 645, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 5 May 2008
Received in revised form 11 August 2008
Accepted 12 August 2008

Keywords:
Anthracnose
In vitro
Nonchemical control
Pathogenicity

ABSTRACT

The objective of this study was to evaluate the antifungal properties of chitosan and to assess its role in the protection of tomato and grape plants against *Colletotrichum* sp. isolated from infected tissues of *Dracaena sanderiana*. The isolate was tested *in vitro* using PDA amended with five concentrations of chitosan (0, 1, 1.5, 2, 2.5%). Chitosan significantly ($P < 0.05$) inhibited the radial growth of this fungus, with a marked effect at the three highest concentrations, after 7 d incubation. The effective concentration that reduced the radial growth to 50% (EC_{50}) was 2.28%. Tomato fruits and single berries treated with aqueous solutions of 1.0 and 2.5% (w/v) chitosan were artificially inoculated with *Colletotrichum* sp. and incubated at 4 and 24 °C. Lesion diameters were recorded 7 and 10 d after inoculation. After 10 d at 24 °C, chitosan significantly ($P < 0.05$) reduced the lesion size of tomato fruits treated with 1.0 and 2.5%. Lesion diameter on pre-treated berries was also significantly reduced at 24 °C. However, no differences were observed between the chitosan concentrations and the corresponding controls at 4 °C; no lesions developed on berries at either 7 or 10 d after inoculation and although lesion size on tomato fruits was smaller for all treatments when stored at 4 °C, there were no treatment differences.

1. Introduction

Colletotrichum spp. are economically important pathogens that cause anthracnose in a wide range of woody and herbaceous crops (Bailey and Jeger, 1992). Symptoms are broad ranging and include stem rot, dieback and seedling blight. Fruits are affected during the pre- and postharvest periods (Sutton, 1992).

Plants of the Agavaceae family (O. Liliales) are susceptible to infections caused by species of *Colletotrichum*. Farr et al. (2006) described three species of this genus restricted to specific genera of Agavaceae: *Colletotrichum agaves*, *Colletotrichum dracaenophilum* and *Colletotrichum phormii*. In addition, *Colletotrichum gloeosporioides* and other *Colletotrichum* species with broad host ranges were included. In the present study an isolate of *Colletotrichum*, obtained from Lucky Bamboo (*Dracaena sanderiana* hort. Sander ex Mast.) plants was used.

Initially, infected stems appeared pale green with yellowish lesions of variable shape and size. The entire stem and petioles progressively became covered with numerous black acervuli, rounded to elongate, with sparse, black setae. Under humid conditions, lesions became covered with gelatinous salmon-coloured spore masses (Fig. 1). A rapid wilting of leaves frequently resulted in the death of the young plants within a 1–2 week period.

Traditionally, the use of synthetic fungicides has been the preferred postharvest treatment to control this microorganism (Aked et al., 2001). However, over time the repeated use of fungicides has resulted in serious problems; the pathogens have developed resistance and residue levels have considerably increased (Mari et al., 2003). Gutierrez et al. (2006) reported that some pathogen species that cause anthracnose may not be sensitive to those fungicides commonly used in tomato production. Latent infection may occur in apparently healthy plants leading to anthracnose associated losses in production yields (McInnes et al., 1992; Mertely and Legard, 2003). Since no chemical disease control methods are available for postharvest diseases in fruit and vegetables, there have been recent efforts over the last year to develop such strategies.

Chitosan, a natural substance derived from chitin, has proved to be effective in preventing fungal growth by directly interfering in or by activating certain biological processes (El Ghaouth et al., 1992a). Chitosan is also known to elicit many plant defense responses by activating pathogenesis-related (PR) gene functions such as chitinases (Mauch et al., 1984; Benhamou and Theriault, 1992), chitosanase, β-glucanases and lignin (Notsu et al., 1994) and callose (Kauss et al., 1989). In tomato, chitosan induces the accumulation of a proteinase inhibitor and when sprayed onto tomato leaves it protects susceptible cultivars against *Alternaria solani* (Ge and Li,

* Corresponding author. Tel.: +34 93 402 14 79.
E-mail address: mmoret@ub.edu (A. Moret).

0261-2194/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2008.08.015

Fig. 1. Stem of *Dracaena sanderiana* exhibiting gelatinous spores from salmon-coloured fungal fruiting bodies (acervuli).

1996). Some authors have reported that chitosan confers protection against *Botrytis cinerea* in *Vitis vinifera* (Romanazzi et al., 2002; Ait Barka et al., 2004; Romanazzi et al., 2006; Nascimento et al., 2007) and Ben-Shalom et al. (2003) indicated that this substance controlled grey mould in cucumber plants. Tomato seeds were also protected against *Fusarium oxysporum* after immersion into a chitosan solution (Borges et al., 2000), and Jia et al. (2007) found that this natural polymer provided effective control of *B. cinerea* and *Penicillium expansum* in tomato fruits. In addition, this substance could potentially provide a protective antifungal coating in post-harvest production (Muzzarelli, 1986; El Ghaouth et al., 1991a; Jiang et al., 2005). Chitosan could also maintain the quality of fruit and vegetables as well as extend their shelf life (El Ghaouth et al., 1992b; Li and Yu, 2000; Han et al., 2004; Wang et al., 2007). This high molecular weight cationic polysaccharide, extracted from the deacetylation exoskeletons of crabs, is a biodegradable and nontoxic polymer. It has the potential to reduce fungal and bacterial infections and could also be used as a substitute for chemicals fungicides.

The objective of this study was to determine the effects of chitosan on the *in vitro* development of *Colletotrichum* sp. obtained from *D. sanderiana* and to evaluate the potential activity of chitosan coating on wounded tomato fruits and berries for the control of *Colletotrichum* anthracnose.

2. Materials and methods

2.1. Fungal isolation

Colletotrichum sp. were isolated from naturally infected plants of Lucky Bamboo (*D. sanderiana*) in the Vegetal Pathology laboratory (Barcelona). The origin of these plants is unknown. Small samples from the side of infected tissues were taken and surface sterilized in 10% sodium hypochlorite (NaOCl) for 5 min, rinsed in sterile water and placed on Petri dishes containing the growth medium potato dextrose agar (PDA, [Sigma–Aldrich, St. Louis, USA] 39 g l^{-1}). Pure cultures were obtained by plating a small piece of the mycelium from the margin of the colony on PDA. They were then incubated at 24 °C for 10 d with a 12-h photoperiod.

2.1.1. The effects of chitosan concentrations on the mycelial growth of Colletotrichum sp.

The effects of chitosan (Sigma–Aldrich, St Louis, U.S.A.) on the mycelial growth of *Colletotrichum* sp. were assessed *in vitro* using PDA amended with five concentrations of this substance (0, 1, 1.5, 2, 2.5%).

The chitosan solution was prepared by dissolving chitosan 85% deacetylated in 0.25 N HCl with continuous stirring at 50 °C. Insoluble material was removed by centrifugation and chitosan was precipitated by neutralization with 1 N NaOH, washed three times with deionized water and air dried (El Ghaouth et al., 1991b). For incorporation into the PDA, purified chitosan was dissolved by stirring in 0.25 N HCl and adjusting the pH to 5.6 using 1 N NaOH. Chitosan solution was added to the medium after autoclaving and poured into 90 mm Petri dishes to yield a total volume of 15 ml per dish. A 5-mm diameter disc from an actively growing PDA culture of *Colletotrichum* was placed fungus-side down in the centre of each Petri dish. Sterile water with the same volume of 0.25 N HCl solution added to all concentrations of chitosan was used as a negative control to check whether it had any effect on the pathogen.

Six replicates of five plates were used for each chitosan concentration. The inoculated plates were incubated at 24 °C for 7 d in the dark. Mycelial growth was determined by measuring colony diameters. The regression line between colony diameter and chitosan concentration was calculated and chitosan sensitivity was determined by calculating 50% effective concentration (EC_{50}).

2.2. Inoculation of fruits

Inoculation was carried out on mature tomato fruits and berries. Tomato fruits were collected from major production zones of the Maresme region (Catalonia), and mature clusters from several grapevines were collected from fields in the Alt Penedès region (Catalonia). The calyx of tomato fruits was removed and both tomato fruits and detached berries were selected based on uniformity of size and absence of visible symptoms on the outside of the fruits. Fruits were surface sterilized in 10% NaOCl for 5 min, followed by two rinses in sterile water and then air dried. Superficial wounds in the epidermis, made with a sterile scalpel, were treated with 15 μl of the chitosan concentrations (1, 2.5%) or sterile distilled water (positive control). For inoculation with the fungus, a 4-mm diameter disc of PDA was removed from the edge of an actively growing culture and placed mycelium-side down on the wound. Fruits inoculated with plugs of sterile PDA were used as negative controls. The inoculated fruits were placed in plastic containers on moist filter paper and incubated at 24 and 4 °C. Twenty tomato fruits and 20 single berries per concentration and temperature were used and the experiment was conducted twice. Differences in the pathogenicity of the *Colletotrichum* isolate in tomato fruits and berries were tested and lesion diameters were measured 7 and 10 d after inoculation. At the end of the experiment, isolations were made from the infected fruits by plating small pieces on PDA and incubating them at 24 °C for fungal identification.

2.3. Statistics

Differences between the concentrations of chitosan on the radial growth of the fungus and the differences between lesion diameters on tomatoes and berries were evaluated by analysis of variance (ANOVA). Fisher's least significance difference (LSD) at $P = 0.05$ was used to compare the radial growth means.

3. Results

3.1. Fungal isolation

Colonies on PDA grew quickly, occupying the whole surface of the Petri dishes within 10 d. They were greyish white to dark grey with pinkish to salmon patches. They were powdery and well

sporulated and had a few tufts associated with fruiting bodies; the production of conidiomata was mainly restricted to the central areas, and the reverse was greyish-brown.

3.2. *Effect of chitosan concentrations on* Colletotrichum *sp. growth* in vitro

The *Colletotrichum* isolate used in this study was progressively inhibited with increasing concentrations of chitosan from 1 to 2.5% (Fig. 2). Fungal growth was significantly affected by all chitosan doses ($P < 0.05$) after 7 d of incubation (Table 1). Concentrations of 2.5 and 2% inhibited the mycelial growth by 63.16 and 39.42%, respectively, compared to the control. The inhibition percentages gradually decreased at 1.5 and 1% with inhibition ratios of 24.95 and 14.96%, respectively. The control solution with hydrochloric acid had no effect on the growth of *Colletotrichum* sp.

The regression line showed the equation $y = -12.906x + 56.155$ ($r^2 = 0.9325$) (Fig. 3) and the EC_{50} was 2.28%.

3.3. *Pathogenicity*

The *Colletotrichum* isolate evaluated in this study was pathogenic to both tomato fruits and berries, however, tomato fruits were more susceptible than single berries. Lesion diameters on the inoculated tomato fruits were larger than those on berries at 24 °C, and at 4 °C no lesions developed in berries, even at the point of the wound. The lesions that developed at 24 °C were dark brown, circular and sunken. They appeared on tomato fruits beneath the inoculated area; the fruit appeared shrivelled. Lesions were delimited by a round, chlorotic halo and the development of soft rot symptoms and the presence of conidiomata were visible 7 d after inoculation (Fig. 4). The symptoms on berries were superficial, no sunken necrotic lesions formed and only abundant aerial whitish mycelia without fruiting bodies were recovered from the lesion (Fig. 5).

3.4. *The effects of chitosan on the inoculated tomato fruits*

On the inoculated tomato fruits, chitosan solutions at 1 and 2.5% significantly reduced ($P < 0.05$) the lesion size at 24 °C 10 d after inoculation. However, significant differences ($P < 0.05$) were only detected between the 2.5% chitosan concentration and the corresponding controls when fruits were stored for 7 d. Anthracnose lesions on fruits maintained at 4 °C were smaller ($P < 0.05$) than on fruits stored at 24 °C and chitosan did not reduce the diameter lesion at the cold storage temperature (Fig. 6).

Table 1
Mycelial growth (mm) of *Colletotrichum* sp. on PDA media supplemented with 0, 1, 1.5, 2, 2.5% chitosan and HCl 0.25 N.

Treatments	Growth (mm)
Chitosan 1%	45.30[a] ± 1.04b[b]
Chitosan 1.5%	39.97 ± 1.58c
Chitosan 2%	32.26 ± 2.13d
Chitosan 2.5%	19.62 ± 1.51e
Chitosan 0%	53.26 ± 2.57a
Control (HCl 0.25 N, pH 5.6)	52.18 ± 1.95a

Colony diameter was determined 7 d after treatment.
[a] Numbers are means ± standard error of 30 replicates.
[b] Means in columns followed by the same letter are not significantly different according to Fisher's least significance difference ($P = 0.05$).

3.5. *Effect of chitosan on the inoculated berries*

After 7 d incubation at 24 °C, inoculated berries treated with 2.5% chitosan solution had smaller and fewer lesions compared to positive controls ($P < 0.05$). Single berries were also more resistant at low temperatures: following storage at 4 °C no lesions developed at either 7 or 10 d after infection (Fig. 7).

Fruits showing symptoms caused by other fungi such as *Rhizopus* spp. or *Alternaria* spp. were removed from the containers to avoid contaminating the other fruits and were excluded from the analysis. No infections occurred on fruits inoculated with sterile PDA (negative controls). Koch's postulates were confirmed after re-isolating the fungi from the infected tissues.

4. Discussion

The present study reveals that the use of chitosan coating on fruits is effective in reducing anthracnose in tomato fruits and berries. Tomato fruits treated with the highest chitosan solutions showed a significant reduction in lesion diameter compared to control plants. There were significant differences between the effects of chitosan concentration and incubation temperature on the lesion size of *Colletotrichum*. Chitosan was most effective when fruits were stored at room temperature. In comparison with controls, the two chitosan concentrations significantly reduced lesion diameters at 24 °C. However, after storage at 4 °C, the anthracnose lesions in the control plants were significantly smaller and, no significant differences in lesion size were observed in plants

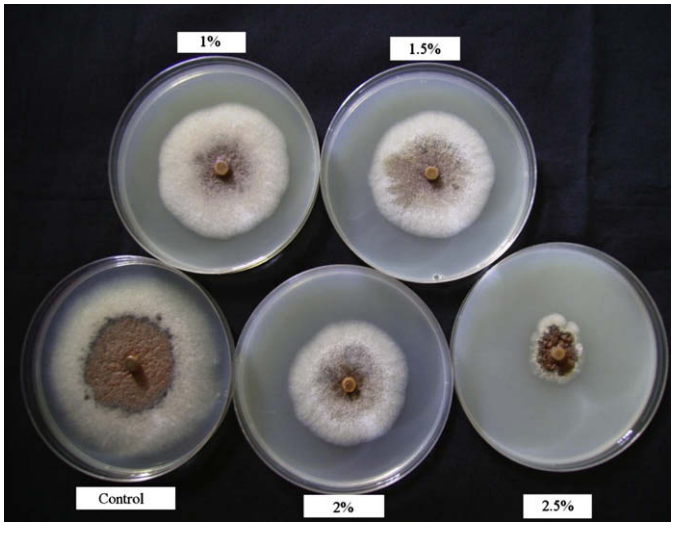

Fig. 2. Mycelial growth (mm) of *Colletotrichum* sp. on PDA supplemented with 0 (control), 1, 1.5, 2 and 2.5% of chitosan. Colony diameter was calculated 7 d after treatment.

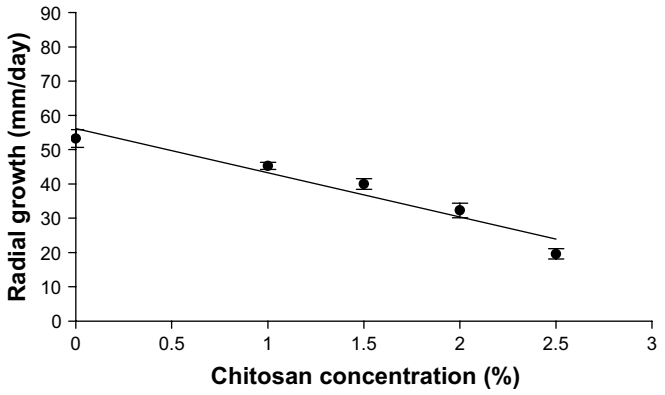

Fig. 3. Linear regression of mean radial growth induced by five chitosan concentrations (0, 1, 1.5, 2, 2.5).

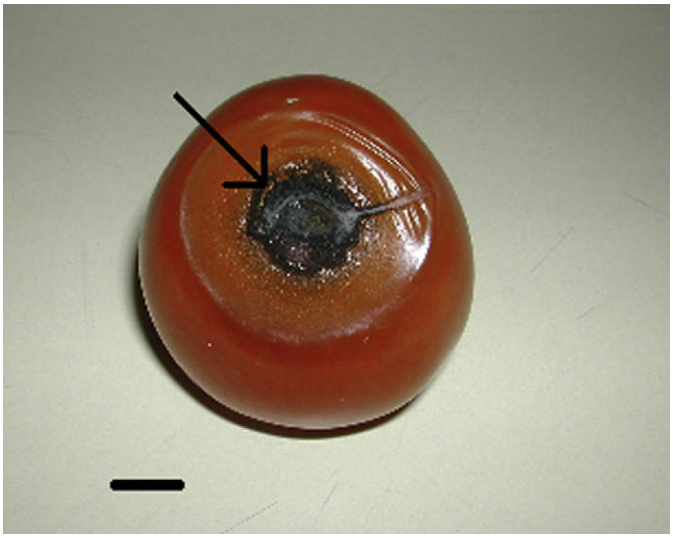

Fig. 4. Symptoms of anthracnose on inoculated tomato fruits. The fruit is shrivelled and the centre of the spot became blackish and exhibited gelatinous spore masses and fungal fruiting bodies (arrow). Bar represents 10 mm.

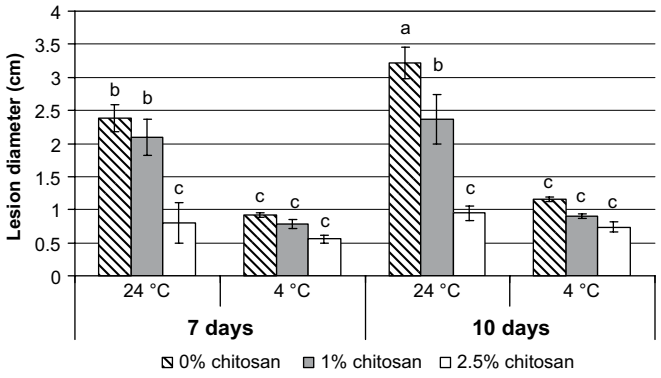

Fig. 6. Fruit lesion diameters (cm) of tomato fruits treated with chitosan and inoculated with *Colletotrichum* sp. 7 and 10 d after inoculation at 4 and 24 °C. Bars with different letters were significantly different according to the LSD test ($P = 0.05$) following ANOVA.

treated with varying doses of chitosan and the controls. Tsai and Su (1999) explained that higher temperatures and acidity in foods increased the bactericidal effect of chitosan.

Grapes were more resistant than tomatoes to the *Colletotrichum* isolate used in this study. When inoculated on tomato fruits, lesions produced by this fungus were larger than those of berries; in some cases no lesions were produced in berries. This could be due to a degree of host specificity of this isolate. Weidemann (1988) found that *Colletotrichum truncatum* was pathogenic in six genera of Leguminosae, but was highly virulent only in *Lathyrus odoratus*, *Vicia ervilia* and *Pisum sativum*. Freeman et al. (2002) demonstrated the host range and specificity of *Colletotrichum acutatum* from strawberries on several plant species.

Previous studies demonstrated that the induction of systemic resistance in plants with natural compounds, including chitosan, is a promising approach to disease control (Gozzo, 2003). Chitosan is an exogenous elicitor whose activity is due to its polycationic structure (Hadwiger et al., 1994) and its receptor is a 78 kDa binding protein (Chen and Xu, 2005). Faoro et al. (2001, 2008) showed that the activity of chitosan was attributed to the accumulation of hydrogen peroxide in treated tissues, which induces a hypersensitive reaction as a consequence of oxidative microburst and phenolic compound deposition. Doares et al. (1995) and Howe (2005) indicate that this substance activates jasmonic acid synthesis in treated hosts. Chitosan oligomers of different molecular weight and degree of acetylation induced an accumulation of phytoalexins in grapevine leaves, which reduced *B. cinerea* and *Plasmopara viticola* infections. Nevertheless, the induction of the defense mechanisms without the antifungal activity was not enough to suppress the disease (Ben-Shalom and Fallik, 2003).

In vitro, chitosan significantly inhibited fungal growth of *Colletotrichum* sp. and significant differences were observed in the mean growth rates of *Colletotrichum* at all concentrations compared to the control. The antifungal activity of chitosan and its ability to reduce the *in vitro* growth of many fungi has been demonstrated in other studies (Allan and Hadwiger, 1979; Romanazzi et al., 2001). Chitosan has been used in numerous industrial and food applications due to its biological and functional properties (Winterowd and Sanford, 1995; No et al., 2007). The experimental data in this study demonstrates that the antimicrobial characteristics of this substance make it a potential, and moreover, a naturally occurring, food coating material. The effectiveness of chitosan has been reported by numerous authors (El Ghaouth et al., 1997; Reddy et al., 2000; Rhoades and Roller, 2000).

Chitosan is nontoxic for humans and has a low environmental impact (Hirano et al., 1990; Li et al., 1992; Shahidi et al., 1999). This is clarified in its recent approval as a food additive in Korea and Japan (Weiner, 1991). The International Commission on Natural Health Products (1995) recognized chitin as a natural product for the 21st Century and in 2005, chitosan was considered as Generally

Fig. 5. Anthracnose symptoms on berries inoculated with *Colletotrichum* sp. and stored at 24 °C. Bar represents 10 mm.

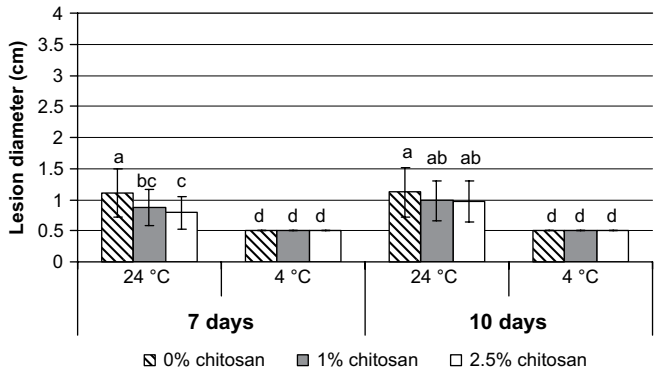

Fig. 7. Lesion diameters (cm) of berries treated with chitosan and inoculated with *Colletotrichum* sp. 7 and 10 d after inoculation at 4 and 24 °C. Bars with different letters were significantly different according to the LSD test ($P = 0.05$) following ANOVA.

Recognized As Safe (GRAS) by the FDA (Food and Drug Administration) based on the scientific procedures for use in foods.

Results show that chitosan offers a safe alternative to synthetic fungicides in postharvest anthracnose diseases and could be considered as a potential agrochemical of low environmental impact.

References

Ait Barka, E., Eullaffroy, P., Clément, C., Vernet, G., 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Rep. 22, 608–614.

Aked, J., Khan, S.H., Magan, N., 2001. Control of the anthracnose pathogen of banana (*Colletotrichum musae*) using antioxidants alone and in combination with thiabendazole or imazalil. Plant Pathol. 50, 601–608.

Allan, C.R., Hadwiger, L.A., 1979. The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp. Mycol. 3, 285–287.

Bailey, J.A., Jeger, M.J. (Eds.), 1992. *Colletotrichum*: Biology, Pathology and Control, Wallingford, UK. 388 p.

Benhamou, N., Theriault, G., 1992. Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Physiol. Mol. Plant Pathol. 41, 33–52.

Ben-Shalom, N., Ardi, R., Pintó, R., Aki, C., Fallik, E., 2003. Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan. Crop Prot. 22 (2), 285–290.

Ben-Shalom, N., Fallik, E., 2003. Further suppression of *Botrytis cinerea* disease in cucumber seedlings by chitosan–copper complex as compared with chitosan alone. Phytoparasitica 31, 99–102.

Borges Jr., A., Borges, A., Gutiérrez, A., Paz-Lago, D., Cabrera, G., Fernández, M., Ramírez, M.A., Acosta, A., 2000. Tomato–*Fusarium oxysporum* interactions: I-chitosan and MSB effectively inhibits fungal growth. Cult. Trop. 21, 13–16.

Chen, H.P., Xu, L.L., 2005. Isolation and characterization of a novel chitosan-binding protein from non-heading Chinese cabbage leaves. J. Integr. Plant Biol. 47, 452–456.

Doares, H., Syrovets, T., Weiler, E.W., Ryan, C.A., 1995. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. Proc. Natl. Acad. Sci. U.S.A. 92, 4095–4098.

El Ghaouth, A., Anil, J., Ponnampalam, R., Boulet, M., 1991b. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. J. Food Process. Preserv. 15, 359–368.

El Ghaouth, A., Arul, J., Grenier, J., Asselin, A., 1992a. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathology 82 (4), 398–402.

El Ghaouth, A., Arul, J., Ponnampalam, R., Boulet, M., 1991a. Chitosan coating effect on storability and quality of fresh strawberries. J. Food Sci. 56 (6), 618–620.

El Ghaouth, A., Ponnampalam, R., Castaigne, F., Arul, J., 1992b. Chitosan coating to extend the storage life of tomatoes. HortScience 27 (9), 1016–1018.

El Ghaouth, A., Wilson, C., Wisniewski, M., 1997. Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases. J. Ind. Microbiol. Biotechnol. 19 (3), 160–162.

Faoro, F., Maffi, D., Cantu, D., Iriti, M., 2008. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. Biocontrol 53, 387–401.

Faoro, F., Sant, S., Iriti, M., Appiano, A., 2001. Chitosan-elicited resistance to plant viruses: a histochemical and cytochemical study. In: Muzzarelli, R.A.A. (Ed.), Chitin Enzymology. Atec, Italy, pp. 57–62.

Farr, D.F., Aime, M.C., Rossman, A.Y., Palm, M.E.M., 2006. Species of *Colletotrichum* on Agavaceae. Mycol. Res. 110, 1395–1408.

Freeman, S., Horowitz, S., Sharon, A., 2002. Survival and host specificity of *Colletotrichum acutatum* from strawberry. Acta Hortic. 567, 619–622.

Ge, Y., Li, D.A., 1996. A molecular study on tomato induced resistance to *Alternaria solani*. In: 8th International Congress of Molecular Plant–Microbe Interaction, Knoxville, TN, USA, pp. 14–19.

Gozzo, F., 2003. Systemic acquired resistance in crop protection: from nature to a chemical approach. J. Agric. Food Chem. 51, 4487–4503.

Gutierrez, L.J., Wang, Y., Lutton, E., McSpadden Gardener, B.B., 2006. Distribution and fungicide sensitivity of fungal pathogens causing anthracnose-like lesions on tomatoes grown in Ohio. Plant Dis. 90, 397–403.

Hadwiger, L.A., Ogawa, O.T., Kuyama, H., 1994. Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. Mol. Plant Microbe Interact. 7, 531–533.

Han, C., Zhao, Y., Leonard, S.W., Traber, M.G., 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus ideaus*). Postharvest Biol. Technol. 33, 67–78.

Hirano, S., Itakura, C., Seino, H., Akiyama, Y., Nonaka, I., Kanbara, N., Kawakami, T., 1990. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem. 38 (5), 1214–1217.

Howe, G.A., 2005. Jasmonates as signals in the wound response. J. Plant Growth Regul. 23, 223–237.

Jia, L., Shiping, T., Xianghong, M., Yong, X., 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Postharvest Biol. Technol. 44, 300–306.

Jiang, Y., Li, J., Jiang, W., 2005. Effects of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature. LWT – Food Sci. Technol. 38, 757–761.

Kauss, H., Jeblick, W., Domard, A., 1989. The degree of polymerization and *N*-acetylation of chitosan determine its ability to elicit callose formation in suspension cell and protoplast of *Catharanthus roseus*. Planta 35, 215–230.

Li, Q., Dunn, E.T., Grandmaison, E.W., Goosen, M.F.A., 1992. Applications and properties of chitosan. J. Bioact. Compat. Polym. 7, 370–397.

Li, H., Yu, T., 2000. Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. J. Sci. Food Agric. 81, 269–274.

Mari, M., Bertolini, P., Pratella, G.C., 2003. Non-conventional methods for the control of postharvest pear diseases. J. Appl. Microbiol. 94, 761–766.

Mauch, F., Hadwiger, L.A., Boller, T., 1984. Ethylene: symptom, not signal for the induction of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. Plant Physiol. 76, 607–611.

McInnes, T.V., Yang, X., Wilson, L.L., 1992. Disease-free plants for management of strawberry anthracnose crown rot. Plant Dis. 76, 260–264.

Mertely, J.C., Legard, D.E., 2003. Detection, isolation, and pathogenicity of *Colletotrichum* spp. from strawberries petioles. Plant Dis. 88 (4), 407–412.

Muzzarelli, R.A.A., 1986. Filmogenic properties of chitin/chitosan. In: Muzzarelli, R.A.A. (Ed.), Chitin in Nature and Technology. Plenum Press, New York, USA, pp. 389–396.

Nascimento, T., Rego, C., Oliveira, H., 2007. Potential use of chitosan in the control of grapevine trunk diseases. Phytopathol. Mediterr. 46, 218–224.

No, H.K., Meyers, S.P., Prinyawiwatkul, W., Xu, Z., 2007. Applications of chitosan for improvement of quality and shelf life of foods. J. Food Sci. 72 (5), 87–100.

Notsu, S., Saito, N., Kosaki, H., Inui, H., Hirano, S., 1994. Stimulation of phenylalanine ammonia-lyase activity and lignification in rice treated with chitin, chitosan and their derivatives. Biosci. Biotechnol. Biochem. 58, 552–553.

Reddy, B.M.V., Belkacemi, K., Corcuff, R., Castaigne, F., Arul, J., 2000. Effect of preharvest chitosan sprays on postharvest infection by *Botrytis cinerea* and quality of strawberry fruit. Postharvest Biol. Technol. 20, 39–51.

Rhoades, J., Roller, S., 2000. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Appl. Environ. Microbiol. 66 (1), 80–86.

Romanazzi, G., Mlikota Gabler, F., Smilanick, J.L., 2006. Preharvest chitosan and postharvest UV radiation treatments suppress gray mold of table grapes. Plant Dis. 90, 445–450.

Romanazzi, G., Nigro, F., Ippolito, A., 2001. Chitosan in the control of postharvest decay of some Mediterranean fruits. In: Muzzarelli, R.A.A. (Ed.), Chitin Enzymology. Atec, Italy, pp. 141–146.

Romanazzi, G., Nigro, F., Ippolito, A., Di Venere, D., Salerno, M., 2002. Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. J. Food Sci. 67 (5), 1862–1866.

Shahidi, F., Arachchi, J.K.V., Jeon, Y.J., 1999. Food applications of chitin and chitosans. Trends Food Sci. Technol. 10, 37–51.

Sutton, B.C., 1992. The genus *Glomerella* and its anamorph *Colletotrichum*. In: Bailey, J.A., Jeger, M.J. (Eds.), *Colletotrichum*: Biology, Pathology and Control, Wallingford, UK, pp. 1–26.

Tsai, G.J., Su, W.H., 1999. Antibacterial activity of shrimp chitosan against *Escherichia coli*. J. Food Prot. 62, 239–243.

Wang, J., Wang, B., Jiang, W., Zhao, Y., 2007. Quality and shelf life of mango (*Mangifera indica* L. cv. *Tainong*) coated by chitosan and polyphenols. Food Sci. Technol. Int. 13 (4), 317–322.

Weidemann, G.J., 1988. Host specificity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and *C. truncatum* in the leguminosae. Phytopathology 78, 986–990.

Weiner, M.L., 1991. An overview of the regulatory status and of the safety of chitin and chitosan as food and pharmaceutical ingredients. In: Brine, C.J., Sandford, P.A., Zikakis, J.P. (Eds.), Advances in Chitin and Chitosan. Proceedings from the 5th International Conference on Chitin and Chitosan, Princetown, New Jersey, USA, pp. 663–672.

Winterowd, J.G., Sanford, P.A., 1995. Chitin and chitosan. Food polysaccharides and their applications. In: Stephen, A.M. (Ed.). Marcel Dekker Inc., New York, pp. 441–462.