Review

Chitosan as an alternative to control phytopathogenic fungi on fruits and vegetables in Mexico

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The control of phytopathogenic fungi has been carried out by synthetic chemical fungicides for many years. However, some studies showed the potential risk they present to the environment and human health. The use of chitosan as an alternative to control phytopathogenic fungi during postharvest is important particularly in Mexico because this polymer exhibits antifungal activity against several important pathogens that affect horticultural commodities. In this review, we present the most relevant scientific studies done in Mexico on the potential use of chitosan as an alternative for the control of phytopathogenic fungi. In addition to its antifungal activity, chitosan forms a semipermeable coating which generates a mechanical barrier against the diffusion of gases that affect the metabolism of agricultural products. The results obtained in different studies are variable and depend on several factors such as the concentration, molecular weight and degree of acetylation of chitosan, as well as on the application method and the storage temperature of the commodities, among others. The studies included in this review may assist to highlight the antifungal potential of chitosan and may spread its use within a sustainable agriculture.

Key words: Chitosan, antifungal effect, phytopathogenic fungi, horticultural commodities, Mexico.

INTRODUCTION

The phytopathogenic fungi cause many diseases on several important agricultural crops. During several years, their control has been based on synthetic chemical fungicides. However, some studies have evidenced the harmful effects of synthetic chemicals on human health and the damage to the environment (Wilson et al., 1999; Northover and Zhou, 2002). Moreover, in several cases it was found that phytopathogenic fungi developed resistance to synthetic chemicals (Förster et al., 2007; Thomdis et al., 2009). Taking these aspects into consideration, some research groups focused their studies toward the evaluation of different alternatives bio-degradable non toxic compounds that exhibit potential to reduce yield losses in agricultural crops. Such compounds include chitosan, a deacetylated derivative of chitin. Chitosan is a polymer composed of β-(1, 4)-2-acetamido-2-deoxy-D-glucose and β-(1, 4)-2-amino-2-deoxy-D-glucose units. The positive charges of chitosan confer its important physiological and biological properties (Rabea et al., 2003). It has been shown that chitosan binds to negatively charged phospholipids of the fungal plasma membrane, which is the basis of its antifungal activity (Palma-Guerrero et al., 2010). Chitosan is commercially obtained in different countries from crustacean wastes in fisheries and food industries. The main sources of chitosan are shrimp, crab and lobster shells (Du et al., 2009; Al-Sagheer et al., 2009). In Mexico, the main source of chitin is shrimp. In addition, a

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potential source to obtain chitin and to reduce production costs are the squid shells (Rocha-Pino et al., 2008). Additionally, chitosan can be obtained with a quality similar to that of commercial chitosan from shrimp exoskeletons in seafood restaurants (Hernández-Cocoletz et al., 2009). The poor solubility of chitosan is the main limiting factor for use, therefore is important to improve this characteristic to enhance the utilization of this polysaccharide. The solubility can be increased significantly by chemical modification of the chitosan chains (Riva et al., 2011) or by reducing the length of the polymer to obtain oligomers of chitosan (degree of polymerization of about 20) which exhibit increased solubility and antifungal activity (Xu et al., 2007).

The antimicrobial properties of chitosan have been proven against several microorganisms such as Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Staphylococcus aureus and Bacillus subtilis (Benhabiles et al., 2012). The antifungal effects of chitosan are related to its deacetylation level, concentration and polymerization grade, among others factors. This polymer has different mechanisms of action to inhibit phytopathogenic fungi and to prevent fungal diseases (Meng et al., 2010). It has been established that chitosan can act at both at an extracellular and at an intracellular level (Hernández-Lauzardo et al., 2011a).

Although it has been considered a polymer with versatile agricultural applications, its antimicrobial activity and mechanisms of action should be thoroughly studied due to the great potential for chitosan application in Latin American agriculture (Lárez-Velázquez, 2008).

In Mexico, some researchers have conducted studies to determine the potential of chitosan in the control of fungal pathogens in pre and postharvest diseases of different commodities. Moreover, the possibility of combining chitosan with essential oils has been reported (Alvarado-Hernández et al., 2011). In general, the proposals focused on the use of environmentally friendly products with antimicrobial properties that may be integrated within sustainable farm management practices. In this review, we report on the most important scientific studies carried out in Mexico on the potential use of chitosan as an alternative for the control of phytopatogenic fungi of horticultural commodities. Thus, we can help to spread the potential benefits of chitosan application for achieving a sustainable agriculture.

*Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill.

*Rhizopus stolonifer* is a very versatile fungus that can grow and develop in different ranges of temperatures (20 to 30°C) and relative humidities (85 to 90%). *R. stolonifer* can colonize the surfaces of vegetable commodities and cause soft rots in a short period of time due to its rapid growth rate. Therefore, this phytopathogen can cause important economic losses at a postharvest stage (Barkai-Golan, 2001). The production and exportation of tomato are very important for the economies of Mexico and Latin America. However, there are reports on significant postharvest losses caused by *R. stolonifer*. About 80% of the total losses reported for tomato fruits are caused by Rhizopus and Alternaria rots (Hahn et al., 2004; Hahn, 2006). There is increasing scientific and public concern for preventing and reducing these losses. Consequently, some studies focused on testing a sensor system for the detection of *R. stolonifer* spores on tomato fruits (Hahn, 2006). On the other hand, some studies have been conducted to evaluate the potential application of chitosan, essential oils and antagonistic microorganisms against *R. stolonifer*. Scientists often report on the necessity to deepen in the knowledge on active compounds, mechanisms of action and antimicrobial properties of these eco-friendly alternatives. Such studies will contribute to the application of effective strategies to control phytopathogenic fungi (Velázquez-del Valle et al., 2008). Different basic and applied studies have been developed to evaluate the effect of chitosan on *R. stolonifer* isolates. In general, the antifungal effect of chitosan on the development of *R. stolonifer* in vitro has been shown regardless of the culture medium used. However, the antifungal effects of chitosan can be better observed by using minimal medium (Guerra-Sánchez et al., 2010). In previous studies, a decline in the membrane integrity of *R. stolonifer* spores was reported in the presence of chitosan (Hernández-Lauzardo et al., 2011b). Also, chitosan of high molecular weight showed the best results to inhibit the infection caused by *R. stolonifer* on tomato fruits. However, the severity of soft rot symptoms was not related with the molecular weight of chitosan, it was evident that infection diminished when fruits were treated with any kind of chitosan (low, medium or high molecular weight) (Hernández-Lauzardo et al., 2012). In other studies on tomatoes fruits, there were no differences in the antifungal activity of chitosan with different grade of polymerization against *R. stolonifer* at a storage temperature of 14°C (Bautista-Baños and Bra vo-Luna, 2004). Hernández-Lauzardo et al. (2008) evaluated the effect of chitosan of low, medium and high molecular weight on three *R. stolonifer* isolates. Their results showed that the low molecular weight chitosan was more effective for inhibition of mycelial growth while the high molecular weight chitosan affected the shape, sporulation and germination of spores. The scanning and transmission electron microscopy studies revealed several and pronounced ridge ornamentations in the chitosan-treated spores. This research may suggest an important hypothesis that relates the molecular weight of chitosan with its effects on hyphae or spores. However, it is necessary to perform further basic studies to clarify the mechanism by which chitosan acts. Recently, the results from some basic studies indicated that chitosan of low, medium and high molecular weight induces the release of intracellular compounds. In addition, it was observed that
consumption of glucose by \textit{R. stolonifer} was notably increased (Guerra-Sánchez et al., 2009). Other study reported a five-fold increase in cell potassium efflux caused by the addition of chitosan to culture medium. No differences on total phospholipids in the plasma membrane were observed. Also, protein content was reduced in about 40\% (Hernández-Lauzardo et al., 2011b). 8\% of the total protein content in the plasma membrane of yeasts corresponds to H\(^+-\)ATPase. This enzyme is a proton pump which has a central function in the regulation of cell homeostasis and several physiological factors have been reported to influence its activity (Portillo, 2000). García-Rincón et al. (2010) reported that chitosan caused an important inhibitory effect on H\(^+-\)ATPase activity in the plasma membrane of \textit{R. stolonifer}. Additionally, other study showed that total H\(^+-\)ATPase activity decreased 52\% in the presence of chitosan. Chitosan treatments decreased the kinetic parameters (\(V_{\text{max}}\) and \(K_{\text{m}}\)) of H\(^+-\)ATPase. Therefore, it was demonstrated that chitosan alters the H\(^+-\)ATPase, affecting the physiological and metabolic functions of \textit{R. stolonifer} (Hernández-Lauzardo et al., 2011b).

Hernández-Lauzardo et al. (2010) suggested that chitosan (at 2.0 mg ml\(^{-1}\)) is a potential alternative for the control of \textit{Rhizopus} decay on peach, papaya and tomato fruits and could be considered as an alternative to control postharvest diseases. Different \textit{R. stolonifer} isolates were obtained from fruits of these three plants and the antifungal effect of chitosan was evaluated on such isolates. Results showed that mycelial growth and sporulation were highly inhibited by all the tested chitosan concentrations. Chitosan at 2.0 mg ml\(^{-1}\) showed the best results. In addition, chitosan was more effective than the synthetic fungicide dichloran in the reduction of the infection percentage of peach (66.20\%), papaya (73.43\%) and tomatoes (33.43\%).

**Colletotrichum gloeosporioides** (Penz.) Penz. and Sacc.

\textit{Colletotrichum gloeosporioides} is the casual agent of the disease known as anthracnose. In Mexico, \textit{C. gloeosporioides} infects a wide range of horticultural hosts such as: mango, avocado, papaya and cherimoya, among others, during the pre and postharvest stages (Gutiérrez-Alonso et al., 2001; Zamora-Magdaleno et al., 2001; Hernández-Albíter et al., 2007; Villanueva-Arce et al., 2008). This fungus produces enzymes that degrade the plant cellular wall (polygalacturonase and pectate-lyase) and causes significant economic losses (Rodríguez-López et al., 2009). Several chemical fungicides are currently being used to control anthracnose. However, there are studies that evidenced that some isolates of \textit{C. gloeosporioides} show resistance to chemical fungicides such as benomyl, thiabendazol and azoxystrobin (Gutiérrez-Alonso and Gutiérrez-Alonso, 2003; Gutiérrez-Alonso et al., 2003). One of the alternatives to chemical control is the use of chitosan. This polymer has been used alone or in combination with extracts from vegetables (cherimoya leaves and papaya seeds) against the development of \textit{C. gloeosporioides} in papaya. Chitosan (2 and 3\%) had a fungicidal effect while chitosan applied in combination with plant extracts had a fungistatic effect. On the other hand, control of anthracnose was achieved when chitosan (1.5\%) was applied (fruits were dipped) for 20 min before the inoculation with \textit{C. gloeosporioides} (Bautista-Baños et al., 2003). In other studies, Bautista-Baños et al. (2005) evaluated the antifungal effect of three different types of chitosan (low, medium and high molecular weight) on two isolates of \textit{C. gloeosporioides} obtained from infected papaya fruit in the states of Veracruz and Guerrero, in Mexico. There were no differences in the fungicidal pattern among the types of chitosan (low, medium and high molecular weight). Nevertheless, the antifungal effect of chitosan varied according to the tested isolate. Overall, sporulation was the most affected variable in the two \textit{C. gloeosporioides} isolates.

**Alternaria alternata** (Fr.: Fr.) Keissl

\textit{Alternaria alternata} attacks several important agricultural crops during pre and postharvest stages. \textit{A. alternata} produces the toxin alternariol and enzymes of cellular maceration (Reddy et al., 2000; Barkai-Golan, 2001). \textit{A. alternata} is reported to be the causal agent of moldy core, the most important disease on apple trees in northern Mexico (Ramirez-Lagarreta et al., 2009). Additionally, this phytopathogenic fungus causes economic losses during the storage of tomato fruits (Félix-Gastélum et al., 2002). Alternatives for the control of \textit{A. Alternata}, such as chitosan, have been proposed. In previous studies, application of chitosan with medium molecular weight at 2.5\% inhibited mycelial growth of \textit{A. alternata} up to 50.6\%. In addition, an inversely proportional relationship was observed between germination of spores and molecular weight of chitosan (Sánchez-Dominguez et al., 2007). In other microscopical studies, Sánchez-Dominguez (2008) observed great damages on fungal cells such as cell disintegration, plasma membrane retraction, a remarkable increase in vacuolization, release of the apical portion of the conidia and lysis of the fungal cells, among other effects. Furthermore, the biochemical studies showed that the phytoalexin rhisitin was not detected from any of the treatments (with or without chitosan). However, other compounds such as alkenes and fatty acids with a known antimicrobial effect were detected. Induction of synthesis of these compounds in the pathosystem \textit{A. alternata}–tomato was associated to the infection process rather than to application of chitosan (Sánchez-Dominguez et al., 2011). In Mexico, \textit{A. alternata} causes important
diseases in agricultural crops. There are few studies involving the application of chitosan for their control. Thus, it becomes necessary to carry out further researches to deepen the knowledge of this polymer and to propose better strategies for its use in agricultural systems.

Other phytopathogenic fungi

Some studies on the antifungal activity of chitosan against other phytopathogenic fungi have been reported. On the other hand, the effect of chitosan alone or in combination with other alternatives such as plant extract or antagonistic microorganisms has also been studied. The antifungal activities of different concentrations of chitosan were evaluated in vitro and in situ against Mucor spp. Their mycelial growth and sporulation were inhibited by the different concentrations of chitosan, the most effective being 2 mg ml⁻¹. The in situ results proved the antifungal potential of chitosan to control postharvest diseases (Hernández-Lauzardo et al., 2007). Recently, the inhibitory properties of middle-viscosity chitosan (133 and 187 kDa) on the growth of Ramularia cercosporellaoides were demonstrated in vitro at different times. At 96 h of incubation, inhibitions of 91.79 and 73.13% of the radial growth of the fungus were observed when 3.4 gL⁻¹ of chitosan was applied. The authors concluded that chitosan could be used to control the disease caused by R. cercosporellaoides on safflower (Quintana-Obregón et al., 2011).

In other study, the antifungal effect of chitosan and plant extracts was evaluated on Fusarium oxysporum and Penicillium digitatum, among others. Chitosan (1.5%) inhibited the mycelial growth and sporulation of F. oxysporum. A synergistic effect was observed only on P. digitatum treated with chitosan and papaya seed extract (Bautista-Baños et al., 2004a). Recently, the antifungal effect of chitosans with low molecular weight and high degree of acetylation combined with the yeast Pichia guillermondii was demonstrated against P. digitatum. The biopolymer and the yeast showed an additive effect, since chitosan was effective in delaying sporulation germination and yeasts in decreasing the apical fungal growth (Pacheco et al., 2008). It is important to determine if application of two or more antifungal compounds helps to enhance the antifungal effects on fungal pathogens. Otherwise, the proposal should be evaluated because it could mean high costs and low benefits.

Application of chitosan to improve the quality of agricultural products

The application of chitosan to increase the quality of agricultural products and extend its shelf life has been studied at the postharvest stage. This polymer forms a semipermeable coating which generates a mechanical barrier against the diffusion of gases (oxygen, dioxide carbon) that affect the metabolism of agricultural products and delayed the loss of fruits firmness, reducing water loss and increasing the content of soluble solids in the final products. Moreover, chitosan was proven to show a high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms. Taking into account these properties, chitosan could be a good alternative to preserve the commodities during storage with no ecological risks (Dutta et al., 2009). Bautista-Baños et al. (2006) observed that chitosan (2.5%) decreases mass loss in plum (Spodias purpurea L.) fruits. In addition, chitosan was evaluated for the control of Penicillium expansum on apples during green-ripe or ripe maturity stages and during storage under ambient or cold temperatures. In general, apples treated with chitosan at 1.0 % showed a lower infection percentage at both maturity stages and storage temperatures (Bautista-Baños et al., 2004b). In other studies, the effects of chitosan alone or in combination with Lactobacillus plantarum were evaluated in rambutan fruit (Nephelium lappaceum). The application of these alternatives contributed to preserve fruit quality characteristics such as firmness, total soluble solids and titratable acidity (Martínez-Castellanos et al., 2009). The titratable acidity is an important parameter that relates to the ripening process of the fruit. By comparison, the coating of mango fruits with chitosan (Mangifer indica L.) did not cause effective results. Coating with chitosan did not reduce the weight loss in mango. Moreover, decreases in firmness and overall quality of fruits were observed. The authors did not observe any significant changes in pH and total soluble solids after 12 days of storage in fruits treated with chitosan (Muy Rangel et al., 2009). The application of chitosan did not influence the content of total soluble solids or the weight loss during storage of papaya fruits. However, a tendency toward more firm fruits was observed when papaya fruits were treated with chitosan combined with papaya seed extract (Bautista-Baños et al., 2003). In Mexico, a few studies on application of chitosan to ornamental plants were reported. For instance, an important research was performed in order to evaluate the effect of chitosan applied alone or combined with hot water to gladiolus (Gladiolus spp.) corms. As a result, germination of corms, number of flowers per spike, number of cormeltes and vase life were positively influenced. The authors suggested that the integration of two alternatives to chemical products may be a suitable option for increasing the quality and vase life of gladiolus plants without negatively affecting the environment (Ramos-García et al., 2009).

CONCLUSIONS

Application of chitosan to fruits and vegetables at a postharvest stage has potential for controlling the phytopathogenic fungi because it is biodegradable, non
toxic and antimicrobial. The use of chitosan as an alternative to control phytopathogenic fungi at a postharvest stage is important, in Mexico in particular, because this polymer has antifungal activity on several important pathogens that affect Mexican horticultural commodities. However, the results obtained in different studies are variable and depend on a number of factors such as the concentration, molecular weight, degree of acetylation, mode of application of chitosan and temperature during storage of the commodities, among others. In summary, it is important to carry out further studies related to the potential application of chitosan for the control of postharvest diseases caused by fungi. At present, there is universal knowledge on the use of chitosan to control organisms that cause diseases in different hosts. The results from the researches mentioned in this work may help to clarify the antifungal spectrum of chitosan and suggest the possibility of its integration into sustainable pre and postharvest management practices for a better, eco-friendly production of fruits and vegetables.

REFERENCES


