# Review paper 10.7251/AGREN2402095E UDC 632.937:632.95 APPLICATION OF CHITOSAN IN THE CONTROL OF ECONOMICALLY IMPORTANT PHYTOPATHOGENIC FUNGI

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# ABSTRACT

Agricultural producers face numerous challenges today. They must strive to achieve high yields to meet the food demands of the population, while also ensuring the safety of the food. Due to the development of resistance in targeted organisms and the adverse effects chemical pesticides pose to human health and the environment, it is necessary to seek eco-friendly methods for pest control. Chitin is the second most abundant biopolymer on earth, after cellulose. Its deacetylated derivative, chitosan, has some beneficial characteristics that give it the potential to become a biopesticide. Phytopathogenic fungi are a limiting factor in agricultural production, causing significant economic losses. Many researchers have confirmed the fungicidal activity of chitosan and it is progressively asserting its role in integrated pest management. It has different modes of action, such as cell membrane/cell wall disruption, interaction with the DNA of microorganisms, chelation of nutrients necessary for microorganisms, film formation and induction of host plant defense mechanism. In vitro studies have shown that chitosan treatment leads to inhibition of mycelial growth, sporulation, and spore germination of phytopathogenic fungi. It also induces morphological alterations in hyphae and spores and influences the production of fungal virulence factors. Chitosan is applied foliarly, to seed, and as a soil enhancer. Extensive research on the synergistic effects of a mixture of chitosan and conventional fungicides shows potential for reducing the usage of chemical pesticides while effectively controlling pathogens.

Keywords: chitin, chitosan, fungicides, phytopathogenic fungi, biopesticides.

#### **INTRODUCTION**

The expanding global population demands intensive agriculture, which requires the extensive application of pesticides. Agricultural producers today face many obstacles. Using synthetic pesticides often leads to negative consequences for the environment and human health. In addition, the intensive and improper application of these chemicals makes it difficult to control the target organisms, through the

development of resistant populations of pests, which creates high production costs and doesn't obtain satisfactory yields. A large number of active substances are prohibited for use, and the possibilities for producers to effectively control pests are reduced. To lower the negative impact and usage of chemical pesticides to a minimum, the research on biological agents in plant protection has increased.

Chitin, a polymer of N-acetyl glucosamine, is the second most abundant polysaccharide in nature, after cellulose. Its limited solubility in water restricts its usage. The characteristics of its deacetylated derivative, chitosan, such as biological activity, non-toxicity to non-target organisms, biodegradability and biocompatibility, make it suitable for use as a biological plant protection agens (Hassan and Chang, 2017).

Research on chitosan as a potential plant protection agent began in the second half of the 20th century. Since then, many papers about the effect of chitosan on various phytopathogenic organisms and pests have been published.

# **CHITIN AND CHITOSAN**

The name chitin originates from the Greek word for armor – *chiton*, because it acts as structural support and protective material for invertebrates. Its role is constructive, it gives strength to the body (holds calcium carbonate in a solid composite structure), and it also serves as a storage of carbohydrates and nitrogen. It is present in the exoskeleton of invertebrates, as well as in the cuticle of insects, and is the main structural polymer in the cell wall of fungi (Crini, 2019).

The history of chitosan begins in 1859 with the work of Charles Rouget (Morin-Crini et al., 2019), who found chitosan as a component of the cell wall of some fungi, such as *Mucor rouxii* and fungi from the *Zygomycetes* class (Janjić, 2010). The name chitosan was introduced in 1894 by Felix Hoppe-Seyler for a chitin derivative soluble in acids, obtained by treating the shells of crabs, scorpions and spiders (Crini, 2019).

Chitosan is a linear polysaccharide, a derivative of chitin with a low degree of acetylation (less than 50 %) (Hromiš, 2015). It consists of 2-amino-2-deoxy- $\beta$ -D-glucopyranose and 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose units interconnected by  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds (Figure 1) (Morin-Crini et al., 2019).



**Figure 1** Chemical structure of chitin (left) and chitosan (right) (Morin-Crini et al., 2019)

Thanks to a series of positive characteristics such as its cationic nature, biodegradability, biocompatibility, biological activity, adhesiveness, as well as

compatibility with other substances, chitosan has been intensively researched in recent decades (Janjić, 2010). Chitosan is biodegradable under the influence of enzymes from various sources, including chitinases, cellulases and hemicellulases, proteases and lipases that are widely distributed in nature, and is considered safe for humans, animals and the environment (Hromiš, 2015). It adheres well to plant surfaces, forming a protective barrier against pests and pathogens (El Hadrami et al., 2010). It can be formulated with other natural compounds or pesticides to improve their effectiveness or provide a controlled release (Maluin et al., 2020).

The largest resource for chitin and chitosan production is biowaste from the marine industry (Morin-Crini et al., 2019). The main producer of chitosan is Japan (Bonecco et al., 2017). In recent years, there has been a growing interest in exploring eco-friendly sources of chitosan, like fungi from various industries and insects (Tyliszczak et al., 2020).

#### **MODE OF ACTION**

Characteristics of chitosan, such as molecular weight and degree of deacetylation, have a direct influence on physical and chemical properties and biological activity. Furthermore, its efficacy is influenced by factors such as the concentration of application, the type of phytopathogenic organism and the host plant species (Orzali et al., 2017).

How chitosan acts on different microorganisms include:

- Cell membrane/cell wall disruption
- Interactions with the DNA of microorganisms
- Chelation of nutrients necessary for the survival of microorganisms
- Film formation on the surface of microorganisms or plant tissue (Korica, 2020)
- Induction of host plant defense mechanism (El Hadrami et al., 2010).

The presence of amino groups in chitosan is responsible for its biological activity. The inhibition of the growth of microorganisms occurs because the cationic amino groups of chitosan bind to the anionic groups of the cell membrane or cell wall of the microorganism (Janjić, 2010). Interactions between the positively charged molecules of chitosan and the negatively charged components of the cell wall of microorganisms can induce depolarization of the biological membrane and inhibition of the exchange of substances or even the rupture of the cell wall and leakage of the cytoplasm (El Hadrami et al., 2010; Korica, 2020).

Low molecular weight chitosan with a higher degree of acetylation showed a better inhibition of phytopathogenic microorganisms, compared to high molecular chitosans (Román-Doval et al., 2023). Also, low molecular weight chitosans are more soluble in water, pass through cell membranes more easily and can interact with the DNA of the microorganism, thereby affecting protein synthesis and mRNA inhibition.

Chitosan can complex metal ions, making them inaccessible to microorganisms (Korica, 2020). This property comes from the high nitrogen content in chitosan (6.89 %) (Román-Doval et al., 2023).

High molecular weight chitosan forms a dense polymer film on the surface of microorganisms. This process effectively hinders the supply of nutrients and oxygen to the microorganism (Korica, 2020). By forming physical barriers at the points of penetration of pathogens, chitosan prevents the spread of pathogens to healthy tissues (El Hadrami et al., 2010).

Elicitors are compounds that can trigger the plants defense mechanisms. These compounds vary in chemical composition and are recognized by receptors within plant cells, they stimulate local or systemic immune responses (Orzali et al., 2017), which are the result of the production of secondary metabolites of the plant. Low molecular weight chitosan induces the formation, activation and accumulation of defense compounds in plants, lignification, ion flux variations, cytoplasmic acidification, membrane depolarization and protein phosphorylation, activation and accumulation of chitinase, glucanase and phenolic compounds, phytoalexin biosynthesis, generation of reactive oxygen species, jasmonic acid biosynthesis and defense gene expression, and inhibition of host maceration enzymes (Bautista-Baños et al., 2006). It induces the formation of callose and proteinase inhibitors in dicotyledonous species (El Hadrami et al., 2010). The plants defense reactions induced by chitosan are influenced by a variety of factors, such as plant species, pathogen species, and the concentration and characteristics of the applied chitosan (Orzali et al., 2017).

Chitosan stimulates the activity of beneficial microorganisms in the soil, including bacteria such as those from the genus *Bacillus* sp., *Pseudomonas fluorescens*, various actinomycetes, mycorrhizal fungi and rhizobacteria (Bell et al., 1998).

#### CHITOSAN AGAINST PHYTOPATHOGENIC FUNGI

The efficacy of chitosan in managing economically important phytopathogenic fungi has been thoroughly examined, in laboratory trials and field conditions. Chitosan is applied foliarly, to seed, and as a soil enhancer.

It is a component of the cell wall of certain types of fungi (Bartnicki-Garcia, 1968). One of the first chitosan screening tests was conducted by Allan and Hadwiger (1979), on fungi with different cell wall compositions. The growth of various species characterized by cell walls containing chitosan (class *Zygomycetes*) remained unaffected, whereas chitosan demonstrated inhibitory effects on pathogens belonging to genera *Phytophtora*, *Rhizoctonia*, *Botrytis*, *Fusarium*, *Verticillium*, *Septoria*, *Helminthosporium*.

Chitosans impact is observable in its ability to inhibit mycelial growth, sporulation, and spore germination, which as a result has a reduction of symptoms and disease spreading. It also induces morphological alterations in hyphae and spores. Furthermore, it influences the production of fungal virulence factors (Orzali et al., 2017).

Due to the significant economic losses that *Botrytis cinerea* can cause, numerous researchers have studied the impact of chitosan on this pathogen, across various plant species and under different conditions. The efficacy of chitosan as a fungicide is influenced by the concentration at which it is applied, as well as the targeted

fungal species. Ghaouth et al. (1992) reported that increasing concentration of chitosan led to enhanced inhibitory effects on the mycelial growth of B. cinerea, Alternaria alternata, Colletotrichum gloeosporioides, while Rhizopus stolonifer showed the least susceptibility to its effects. Observing B. cinerea and R. stolonifer on strawberries, Ghaouth et al. (1992) examined the impact of chitosan on spore germination and radial growth of these phytopathogenic fungi, as well as its potential to reduce the strawberry deterioration caused by infection with these pathogens. Chitosan, when applied at concentrations ranging from 0.75 to 6.0 mg/ml, effectively inhibited the spore germination and radial growth of both fungi. Notably, its inhibitory effect was more apparent on B. cinerea compared to R. stolonifer. Complete inhibition was not attained, suggesting that chitosan displayed fungistatic rather than fungicidal activity. Chitosan disrupts the fungal cell wall, leading to its thinning and inducing the leakage of amino acids and proteins from cells of both B. cinerea and R. stolonifer within one hour after treatment. In addition, at a concentration higher than 1.5 mg/ml, this biopolymer induced excessive hyphal branching of R. stolonifer. Also, Alfaro-Gutiérrez et al. (2014) stated that treating of R. stolonifer with chitosan led to various morphological and physiological changes, such as branching, abnormal shape and swelling of hyphae. Cheah et al. (1997) reported similar conclusions about hyphal morphology, when microscopically examining the mycelium of Sclerotinia sclerotiorum after treatment with chitosan. They found that it was twisted and branched, compared to the mycelium in the control. Ben-Shalom et al. (2003) stated nearly complete inhibition of conidia germination of B. cinerea when applying chitosan at a concentration of 50 ppm under in vitro conditions. Furthermore, chitosan suppressed the elongation of germ tube. Microscopic observation of Ait Barka et al. (2004) revealed that chitogel induces morphological and structural alterations in the hyphal cells of B. cinerea. In vitro experiments demonstrated that chitosan inhibited spore germination, germ tube elongation, and mycelium growth of this pathogen. Additionally, it caused damage to the spore plasma membrane (Liu et al., 2007). This derivative of chitin induces changes in the spore morphology of C. gloeosporioides at a concentration of 1.5 %, seven hours after incubation (Bautista-Baños et al., 2003). Also, it influenced aggregation and induced abnormal cell shape, as well as hyphal branching and twisting of A. alternata (Reddy et al., 1997). In addition, it led to spore aggregation and morphological changes of the hyphae, which included swelling, polarization and the appearance of germ tubes of Aspergillus niger (Plascencia-Jatomea et al., 2003).

Some researchers have examined the effect of chitosan film on the decay of various fruits. Chitosan film applied to strawberries at a concentration of 15 mg/ml had efficacy in reducing strawberry decay, caused by *B. cinerea* and *R. stolonifer*, by over 60 % compared to control (Ghaouth et al., 1992). Du et al. (1997) studied the impact of chitosan film on the preservation of peaches. The application of this biopolymer coating resulted in a reduction in the occurrence and growth of *B. cinerea*. At the end of storage, the decayed area was smaller compared to the control. Chitosan significantly inhibited the growth of *B. cinerea* in Petri dishes.

After 3 days of culture, the colony diameter of this pathogen on KDA medium containing 0, 0.05 and 0.20 % chitosan was 77.4, 65.4 and 25.6 mm, respectively. Cheah et al. (1997) examined the effect of a chitosan film at concentrations of 2 and 4 % on carrot roots. Five days after the treatment, the disease intensity caused by *S. sclerotiorum*, decreased compared to control.

The timing of chitosan treatment can also influence its effectiveness against pathogens. Experiments assessing the efficacy of chitosan applied both pre and post-harvest, revealed that all applied concentrations of chitosan led to a reduction in the occurrence of gray mold of table grapes. Post-harvest treatments comprised immersing grape bunches or berries in chitosan solutions of 0.1, 0.5, and 1 %, followed by inoculation with a pathogen spore suspension. Chitosan solutions at concentrations of 0.5 and 1.0 % exhibited a significantly reduced percentage of infected bunches or berries and a smaller surface area of lesions compared to the control (Romanazzi et al., 2002). Treating with chitosan one hour before inoculation with conidia of pathogen resulted in a 65 % reduction in gray mold occurrence. Treating four or twenty-four hours before spore inoculation reduced disease development by 82 and 87 %, respectively. Treating one hour after inoculation had a slightly weaker effect, reducing the occurrence of the disease by 52 % (Ben-Shalom et al., 2003). Also, de Capdeville et al. (2002) examined the effect of chitosan on *Penicillium expansum*, the causal agent of blue mold, on the apple variety Crveni delišes. Fresh and refrigerated fruits were treated with chitosan, and pathogen inoculation was performed 24, 48 and 96 hours after treatment. Chitosan was found to be effective in controlling the development of the pathogen. However, it was observed that fresh apples exhibited a more favorable response to the treatment compared to those from cold storage. Ait Barka et al. (2004) examined the influence of chitosan in gel form, incorporated into a medium for cultivating vine shoots, on the growth of B. cinerea. They confirmed that the increase in concentration of chitogel was associated with an increase in pathogen inhibition. When shoots were inoculated with B. cinerea, the characteristic symptoms of gray mold were reduced. The ability of a chitogel-modified medium to inhibit pathogen growth may imply systemic distribution of the chitosan throughout the plant. As stated by Liu et al. (2007), the impact of chitosan on B. cinerea and P. expansum in tomato fruits may be due to a direct fungicidal effect or induction of the defense mechanism of the host plant. C. gloeosporioides shows high sensitivity to the effects of chitosan, as it impacts mycelial growth even at a concentration of 0.5 %. At concentrations of 2.0 and 3.0 %, chitosan completely inhibits the mycelium growth of this fungi (Bautista-Baños et al., 2003).

Hernández-Lauzardo et al. (2008) examined the impact of three chitosans with varying molecular weights on the development of three isolates of R. *stolonifer*. The findings indicated that chitosan with low molecular weight was more effective in inhibiting mycelial growth, whereas high molecular weight chitosan influenced sporulation, spore shape, and germination. Similar results were obtained by Alfaro-Gutiérrez et al. (2014) by treating R. *stolonifer* with oligochitosan, which led to the inhibition of hyphal development. Application of these compounds also resulted in

a significant thickening of the fungus cell wall, increasing it by two to three times. Furthermore, at low concentrations of these compounds, the respiration of R. stolonifer is stimulated. The impact of various concentrations of chitosan on the growth and toxin production of A. alternata, the causal agent of black spot of tomato, was evaluated by integrating chitosan into the PDA medium at concentrations of 100, 200, 400, 800, 1600, and 3200 µl/ml. After 15 days of incubation, an assessment was conducted. The study concluded that chitosan inhibits the growth and toxin production of this pathogen at higher concentrations. Additionally, it was found that at lower concentrations, the effect of chitosan on toxin production is stronger than on mycelial growth (Reddy et al., 1997). Cheah et al. (1997) studied the impact of three concentrations of chitosan (1, 2, and 4 %) on the mycelial growth of S. sclerotiorum in vitro. A chitosan concentration of 4 % demonstrated the highest efficacy in inhibiting mycelial growth. Xu et al. (2007) conducted experiments to evaluate the fungicidal activity of oligochitosan against various phytopathogenic fungi in laboratory tests. They showed that *Phytophtora* capsici exhibited the highest sensitivity among all the tested fungi, which included B. cinerea, Colletotrichum orbiculare, Exserohilum turcicum, Fusarium graminearum, Fusarium oxysporum, Verticillium dahliae. Additionally, they found that oligochitosan had a better inhibitory effect on mycelial growth than chitosan. Plascencia-Jatomea et al. (2003) studied the effect of chitosan and temperature on the germination of A. niger spores. Chitosan applied at a concentration of 3 g/l led to the inhibition of the radial growth of mycelia by 73 % after 24 hours, while the percentage of inhibition of spore germination after 13 hours was 40 %. The inhibitory effect of chitosan was stronger at temperatures below 18 °C. Chitosan reacts with DNA of A. niger, affects protein synthesis and leads to mRNA inhibition (Sebti et al., 2005).

Incorporated into the soil, chitosan reduces the occurrence of diseases caused by Fusarium sp., Alternaria solani and Aspergillus flavus. Its effectiveness is presumed to emerge from its ability to stimulate beneficial microorganisms in the soil, such as Bacillus sp., P. fluorescens, actinomycetes, mycorrhizae and rhizobacteria. These organisms can directly affect phytopathogenic organisms, or they can enhance the plants resistance by improving its defense mechanisms (Orzali et al., 2017). Chitosan has the potential to become an important agent in the control of soilborne pathogens in greenhouse production. Applied at a concentration of 37.5 mg/l in the plant growth medium, chitosan significantly reduced plant death, root rot symptoms and yield losses caused by Fusarium oxysporum f. sp. radicis-lycopersici on tomatoes grown in a greenhouse. Cytological research revealed that the mechanism of action involves inducing resistance in the host plant against colonization by this pathogen (Lafontaine and Benhamou, 1996). In general, the sporulation of fungi treated with chitosan is reduced compared to the control. Bautista-Baños et al. (2004) examined the inhibition of mycelial growth of three pathogens: F. oxysporum, Penicillium digitatum and R. stolonifer in vitro. Chitosan concentration of 1.5 % effectively suppressed the mycelial growth of *Rhizopus* and *Penicillium* species. Moreover, a

0.5 % chitosan solution notably decreased the sporulation of F. oxysporum, while a 1.5 % chitosan solution similarly reduced the sporulation of R. stolonifer. However, there are cases when the sporulation is enhanced. P. digitatum showed significantly higher sporulation with both concentrations, indicating a stimulating effect probably due to the stressful conditions induced by chitosan. Examination of the biological activity of chitosan on Pythium aphanidermatum affecting cucumber plants cultivated in substrates containing either 100 or 400 µg chitosan/ml reaffirmed its efficacy in controlling rot induced by this phytopathogenic fungus. It triggered various defense mechanisms within the host plant, including the development of structural barriers in root tissue and the stimulation of antifungal hydrolases such as chitinases, chitosanases, and  $\beta$ -1.3 chitinases in both roots and leaves. Observation of fungal hyphal cells revealed that chitosan induced cell wall relaxation, vacuolation, and, occasionally, protoplasm disintegration. This may partially elucidate the pathogens limited ability to colonize root tissue in the presence of chitosan. The combined effect of chitosans fungicidal activity and its induction of the defense mechanism make it a promising agent for controlling this pathogen (Ghaouth et al., 1994). Some researchers compared the fungicidal action of chitosan and its derivatives. Rabea and Steurbaut (2010) tested eight chemically modified chitosans on A. alternata, F. oxysporum and Pythium debaryanum. N-(pfluorobenzyl) chitosan showed the strongest inhibitory activity on the growth of A. alternata. The most effective in inhibiting F. oxysporum and P. debaryanum was N-(o-chloro,o-fluorobenzyl) chitosan. In general, chemically modified chitosans showed a stronger effect on the germination of spores of phytopathogenic fungi compared to unmodified ones.

Seed treatment provides protection and enhances plant development during the initial stages of growth (Orzali et al., 2017). Chitosan increases the content of proline and sugar in seeds, which changes the permeability of the plasma membrane. In addition, it improves enzyme activity (Román-Doval et al., 2023). High molecular weight chitosan possesses biopolymer characteristics and can be applied to seeds forming a film that effectively shields against pathogen infections. In the seed industry, treatment with chitosan consists of immersing the seeds in a chitosan suspension (up to 4 %), followed by drying. Chitosan layers reduce the number of fungi present on the seeds and promote plant growth. The molecular weight of chitosan, the presence of surfactant, the pH value, as well as the thickness of chitosan layers on the seed are parameters that affect seed germination, as well as fungicidal activity and vegetative growth (El Hadrami et al., 2010). Treating wheat seeds with chitosan, for protection against F. graminearum, led to an increase in yield by 20 %, alongside enhancements in seed quality and germination potential (Reddy et al., 1999). Attjioui et al. (2021) showed strong synergistic effects of chitosan polymer and oligomers in inhibiting conidia germination and fungal growth of F. graminearum. Chitosan is also used as a protective coating on corn seeds to protect against Fusarium moniliforme and A. flavus (Román-Doval et al., 2023).

Mazaro et al. (2009) treated tomato and sugar beet seeds by immersing them in a chitosan suspension. The seeds were subsequently planted in containers containing substrate infected with *Rhizoctonia* sp. and placed in a greenhouse for 14 days. Chitosan enhanced seed resistance against this pathogen, elevating the activity of phenylalanine ammonia-lyase while affecting the total protein content and total and reducing sugars in the leaves.

Chitosan has also found application as a carrier for various active substances, such as essential oils, protecting them from various environmental factors and thus extending their persistence. By applying a film of chitosan and essential oil to wheat seeds, the development of F. graminearum was reduced compared to the control, without a negative effect on germination and plant development in the initial stages. In addition, chitosan film on corn and wheat seeds increased seed germination rate and stress tolerance. Chitosan diffuses through seed integuments, affects cell metabolism and thus induces a series of plant defense reactions (Orzali et al., 2017).

### SYNERGISTIC EFFECT OF CHITOSAN AND FUNGICIDES

In recent years, a lot of research has been conducted on the synergistic effect of chitosan and conventional fungicides. This approach holds promise for reducing chemical pesticide usage while effectively controlling pathogens. Chitooligosaccharides enhance the effectiveness of synthetic fungicides. This was observed with B. cinerea on strawberries and Venturia inaequalis on apples, under both in vitro and in vivo conditions. The researchers studied both the separate and combined effects of chito-oligosaccharides and synthetic fungicides, which included active ingredients such as fenhexamide, cyprodinil and fludioxonil, azoxystrobin, boscalid and pyraclostrobin, and dithianon. The most significant synergistic effect was observed on *B. cinerea*. When applied individually at a lower concentration, a fungicide containing boscalid and pyraclostrobin, as well as chitooligosaccharide, did not significantly inhibit spore germination. However, when combined, they reduced spore germination by 90 %. Previous research suggests that chito-oligosaccharide increases the membrane permeability of fungi, which allows fungicides to reach their site of action more quickly (Rahman et al., 2014). Le et al. (2019) found that a mixture of silver-incorporated chitosan nanocomposites and fungicide propineb exhibit notably enhanced antifungal efficacy against P. capsici compared to each component. Lemke et al. (2022) showed that a mixture of low molecular weight chitosan and copper acetate act synergistically and enable a 50 % reduction in copper concentration while still retaining the antifungal effectiveness against F. graminearum. Chitosan combined with copper fungicides, is more effective against Plasmopara viticola than copper alone (Romanazzi et al., 2024). These authors conducted a three-year-long experiment in three vineyards under different environmental conditions. They applied 0.5 % chitosan alone, in rotation with copper and combination with copper, and also conventional fungicides without chitosan. The control stayed untreated. Chitosan treatments consistently decreased the grapevine downy mildew

McKinney Index on both leaves and bunches. When applied alone, chitosan exhibited lower efficacy compared to copper. However, after copper it had similar effectiveness. In combination with copper, it had greater efficacy than the copper standard. Ippólito et al. (2017) found that the combination of low, ineffective doses of mancozeb with chitosan successfully suppresses *Phytophtora infestans* in potatoes. It is presumed that such efficacy is a result of the fungicidal activity of chitosan and its ability to induce the plants defense mechanisms.

### **CONCLUSION**

Based on numerous studies, it can be concluded that chitosan has good activity against many plant pathogens. This polymer has been established as a notable inducer of plant resistance. Hence, biopesticides based on chitosan hold the potential to supplement conventional pesticides in integral and conventional plant protection. This can be the effective management of control of pathogens to minimize the negative impacts of chemical pesticides. However, the effectiveness of chitosan-based biopesticides depends on numerous factors, such as the species of phytopathogenic organism, chemical characteristics of chitosan, application method and concentration, and environmental conditions. Indeed, we can expect that this natural substance will occupy a significant place in the future for controlling harmful agents in agricultural production.

#### REFERENCES

- Ait Barka, E., Eullaffroy, P., Clément, C., & Vernet, G. (2004). Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Reports*, 22(8), 608–614.
- Alfaro-Gutiérrez, I. C., Guerra-Sánchez, M. G., Hernández-Lauzardo, A. N., & Velázquez-del Valle, M. G. (2014). Morphological and Physiological Changes on *Rhizopus stolonifer* by Effect of Chitosan, Oligochitosan or Essential Oils. *Journal of Phytopathology*, 162(11–12), 723–730.
- Allan, C. P., & Hadwiger, L. A. (1979). The Fungicidal Effect of Chitosan on Fungi of Varying Cell Wall Composition. *Experimental Mycology*, 3
- Attjioui, M., Gillet, D., El Gueddari, N. E., & Moerschbacher, B. M. (2021). Synergistic Antimicrobial Effect of Chitosan Polymers and Oligomers. *Molecular Plant-Microbe Interactions*, 34(7), 770–778.
- Bartnicki-Garcia, S. (1968). Cell Wall Chemistry, Morphogenesis, and Taxonomy of Fungi. *Annual Review of Microbiology*, 22(1), 87–108.
- Bautista-Baños, S., Hernández-Lauzardo, A. N., Velázquez-Del Valle, M. G., Hernández-López, M., Ait Barka, E., Bosquez-Molina, E., & Wilson, C. L. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection*, 25(2), 108–118.
- Bautista-Baños, S., Hernández-López, M., & Bosquez-Molina, E. (2004). Growth Inhibition of Selected Fungi by Chitosan and Plant Extracts. *Revista Mexicana de Fitopatología*, 22(2), 178–186.
  - 104

- Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., & Wilson, C. L. (2003). Effects of chitosan and plant extracts on growth of *Colletotrichum* gloeosporioides, anthracnose levels and quality of papaya fruit. Crop Protection, 22(9), 1087–1092.
- Bell, A. A., Hubbard, J. C., & Liu, L. (1998). Effects of Chitin and Chitosan on the Incidence and Severity of Fusarium Yellows of Celery. *Plant Disease*, 82(3), 322–328.
- Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C., & Fallik, E. (2003). Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan. *Crop Protection*, 22
- Bonecco, M. B., Martínez Sáenz, M. G., & Buffa, L. M. (2017). Chitosan, from residue to industry. Advances in physicochemical properties of biopolymers. Bentham e-books, 224–256.
- Cheah, L. H., Page, B. B. C., & Shepherd, R. (1997). Chitosan coating for inhibition of sclerotinia rot of carrots. *New Zealand Journal of Crop and Horticultural Science*, 25(1), 89–92.
- Crini, G. (2019). Historical review on chitin and chitosan biopolymers. Environmental Chemistry Letters 2019 17:4, 17(4), 1623–1643.
- de Capdeville, G., Wilson, C. L., Beer, S. V., & Aist, J. R. (2002). Alternative Disease Control Agents Induce Resistance to Blue Mold in Harvested 'Red Delicious' Apple Fruit. *Phytopathology*®, 92(8), 900–908.
- Du, J., Gemma, H., & Iwahori, S. (1997). Effects of Chitosan Coating on the Storage of Peach, Japanese Pear, and Kiwifruit. J. Japan. Soc. Hort. Sci, 66(1).
- El Hadrami, A., Adam, L. R., El Hadrami, I., & Daayf, F. (2010). Chitosan in Plant Protection. *Mar. Drugs*, 8, 968–987.
- Ghaouth, A. El, Arul, J., Grenier, J., & Asselin, A. (1992). Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. *Phytopathology*, 82(4), 398–402.
- Ghaouth, A. El, Arul, J., Grenier, J., Benhamou, N., Asselin, A., & Belanger, R. (1994). Effect of chitosan on cucumber plants: Suppression of Pythium aphanidermatum and induction of defense reactions. *Phytopathology*, 84(3), 313–320.
- Hassan, O., & Chang, T. (2017). Chitosan for Eco-friendly Control of Plant Disease. *Asian Journal of Plant Pathology*, 11(2), 53–70.
- Hernández-Lauzardo, A. N., Bautista-Baños, S., Velázquez-del Valle, M. G., Méndez-Montealvo, M. G., Sánchez-Rivera, M. M., & Bello-Pérez, L. A. (2008). Antifungal effects of chitosan with different molecular weights on in vitro development of *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. *Carbohydrate Polymers*, 73(4), 541–547.
- Hromiš, N. (2015). Razvoj biorazgradivog aktivnog ambalažnog materijala na bazi hitozana: sinteza, optimizacija svojstava, karakterizacija i primena. Doktorska disertacija. Univerzitet u Novom Sadu.
- Ippólito, S. D., Mendieta, J. R., Terrile, M. C., Tonón, C. V., Mansilla, A. Y., Colman, S., Albertengo, L., Rodríguez, M. S., & Casalongué, C. A. (2017).

Chitosan as Source for Pesticide Formulations. *Biological Activities and Application of Marine Polysaccharides*.

- Janjić, S. (2010). Prilog proučavanju dobijanja hemijskih dvokomponentnih vlakana specijalnih svojstava na bazi polisaharida. Doktorska disertacija. Univerzitet u Beogradu.
- Korica, M. D. (2020). Dobijanje bioaktivnih nanostrukturnih materijala na bazi celuloze i hitozana. Doktorska disertacija. Univerzitet u Beogradu.
- Lafontaine, P. J., & Benhamou, N. (1996). Chitosan treatment: An emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici. Biocontrol Science and Technology*, 6(1), 111–124.
- Le, V. T., Bach, L. G., Pham, T. T., Trang Le, N. T., Phan Ngoc, U. T., Nguyen Tran, D.-H., & Nguzen, D. H. (2019). Synthesis and antifungal activity of chitosan-silver nanocomposite synergize fungicide against *Phytophthora capsici. Journal of Macromolecular Science, Part A*, 56(6), 522–528.
- Lemke, P., Jünemann, L., & Moerschbacher, B. M. (2022). Synergistic Antimicrobial Activities of Chitosan Mixtures and Chitosan–Copper Combinations. *International Journal of Molecular Sciences*, 23(6).
- Liu, J., Tian, S., Meng, X., & Xu, Y. (2007). Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biology and Technology*, 44(3), 300–306.
- Maluin, F. N., Hussein, M. Z., Azah Yusof, N., Fakurazi, S., Idris, A. S., Zainol Hilmi, N. H., & Jeffery Daim, L. D. (2020). Chitosan-Based Agronanofungicides as a Sustainable Alternative in the Basal Stem Rot Disease Management. *Journal of Agricultural and Food Chemistry*, 68(15), 4305–4314.
- Mazaro, S. M., Wagner Júnior, A., Santos, I. dos, Citadin, I., Possenti, J. C., & Gouvêa, A. de. (2009). Control of beet and tomato damping-off by seed treatment with chitosan. *Pesquisa Agropecuária Brasileira*, 44(11), 1424–1430.
- Morin-Crini, N., Lichtfouse, E., Torri, G., & Crini, G. (2019). Fundamentals and Applications of Chitosan, 49–123.
- Orzali, L., Corsi, B., Forni, C., & Riccioni, L. (2017). Chitosan in Agriculture: A New Challenge for Managing Plant Disease. In *Biological Activities and Application of Marine Polysaccharides*.
- Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M. M., & Shirai, K. (2003). Effect of Chitosan and Temperature on Spore Germination of Aspergillus niger. *Macromolecular Bioscience*, 3(10), 582–586.
- Rabea, E. I., & Steurbaut, W. (2010). Chemically Modified Chitosans as Antimicrobial Agents against Some Plant Pathogenic Bacteria and Fungi. In *Plant Protect. Sci*, 46(4).
- Rahman, M. H., Shovan, R., Hjeljord, L. G., Aam, B., Eijsink, V. G. H., Sørlie, M.,
  & Tronsmo, A. (2014). Inhibition of Fungal Plant Pathogens by Synergistic Action of Chito-Oligosaccharides and Commercially Available Fungicides. *Plos one*, 9(4)

- Reddy, M. V. B., Arul, J., Angers, P., & Couture, L. (1999). Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *Journal of Agricultural and Food Chemistry*, 47(3), 1208–1216.
- Reddy, M. V. B., Barka, E. A., Castaigne, F., & Arul, J. (1997). Effect of Chitosan on Growth and Toxin Production by *Alternaria alternata* f. sp. *lycopersici*. *HortScience*, 32(3), 467F–468.
- Romanazzi, G., Nigro, F., Ippolito, A., DiVenere, D., & Salerno, M. (2002). Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. *Journal of Food Science*, 67(5), 1862–1867.
- Romanazzi, G., Piancatelli, S., Potentini, R., D'ignazi, G., & Moumni, M. (2024). Applications of chitosan alone, alternated or combined with copper for grapevine downy mildew management in large scale trials. *Journal of Cleaner Production*
- Román-Doval, R., Torres-Arellanes, S. P., Tenorio-Barajas, A. Y., Gómez-Sánchez, A., & Valencia-Lazcano, A. A. (2023). Chitosan: Properties and Its Application in Agriculture in Context of Molecular Weight. *Polymers*, 15(13), 2867.
- Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S., & Coma, V. (2005). Chitosan Polymer as Bioactive Coating and Film against Aspergillus niger Contamination. M100 Journal of Food Science, 70(2)
- Tyliszczak, B., Drabczyk, A., Kudłacik-Kramarczyk, S., & Sobczak-Kupiec, A. (2020). Sustainable Production of Chitosan. *Sustainable production: Novel trends in energy, environment and material systems*, 45–60.
- Xu, J., Zhao, X., Han, X., & Du, Y. (2007). Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. *Pesticide Biochemistry and Physiology*, 87(3), 220–228.