

SIPCO HYSHIELD – Benefits of Chitosan for Agricultural Uses



Summary

Chitosan is an active compound derived from crustaceans that finds many possible applications in agriculture and other industries. Demonstrated benefits of Chitosan application as drench or foliar spray in crop production include:

- ✓ Improved yield due to direct effects on plant nutrition and plant growth stimulation;
- ✓ Toxicity to plant pests (insects, pathogenic nematodes) and pathogens (fungal, bacterial, viral);
- ✓ Elicitation of plant defense mechanisms;
- ✓ Stimulation of growth and activity of beneficial microbes.

Chitosan is a viable, natural alternative to traditional agricultural pesticides and fertilizers.

What is Chitosan?

Chitin is the main component of the exoskeleton of arthropods (invertebrates) such as shrimp, crab, lobster, insects, mites and spiders. It is also one of the major constituents of fungal cell walls. Chitin is one of the most abundant organic polymers found in nature (second to cellulose). Chitosan is the deacetylated form of chitin, which is typically produced by treating shrimp shells with an alkaline substance. Chitosan is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 4)-linked D-glucosamine and N-acetyl-D-glucosamine.

Agricultural Uses of Chitosan

Chitosan has a number of commercial and possible biomedical uses. In agriculture, chitosan is typically used as a natural seed treatment and plant growth enhancer, and as an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections [1].

The agricultural and horticultural uses for chitosan, primarily for plant defense and yield increase, are based on how this glucosamine polymer influences the biochemistry and molecular biology of the plant cell. The cellular targets are the plasma membrane and nuclear chromatin. Subsequent changes occur in cell membranes, chromatin, DNA, calcium, MAP Kinase, oxidative burst, reactive oxygen species, callose pathogenesis-related (PR) genes and phytoalexins [2].

In addition to increases in photosynthesis and vegetative growth, chitin-based treatments have been shown to modify developmental processes. The germination of seeds has been shown to be improved in a range of crops following chitin-based treatments including maize [3] and wheat [4].

Chitin, and all its derivatives, have a high nitrogen content of 6.1%–8.3% [5]. This is a comparable level to other organic fertilizers such as dried blood, bone meal, and hoof and horn meal [6]. While chitin has a high thermal and chemical stability [5], it can quickly be utilized as both a nitrogen source and energy source by plants and microbes when added to crops. Plants can access the nitrogen in chitin via microbial breakdown and the release of inorganic nitrogen, or directly taking up monomers as organic nitrogen [7, 8]. Spiegel *et al.* [7] demonstrated that Chinese cabbages treated with chitin-based products grew faster than plants treated with a standard mineral fertilizer.

Partially purified chitin also has promise in plant nutrition as it can be used to add organic matter to soils without raising the C:N ratio. In addition to supplying nitrogen, the exoskeletons of crustaceans from which chitosan is commercially extracted are also high in calcium minerals, therefore, chitin-based products that have only been partially purified will also contain substantial levels of calcium, an important macronutrient [9].

The cationic properties of chitosan also make it suitable as a medium for supplying additional essential nutrients. The functional hydroxyl and amino groups on deacetylated chitosan allow the formation of coordination compounds (complexes) with ions of copper, zinc, iron and others, but not with those of alkaline metals (e.g. potassium) or alkaline earth metals (e.g. calcium or magnesium) [10]. This makes chitosan a sustainable alternative to synthetic chelation agents, such as EDTA that are routinely used to deliver iron and other nutrients to overcome their poor solubility in calcareous/neutral soils [11].

Chitosan can also form gels that absorb substantial volumes of water due to its high molecular weight, and porous structure [12]. These “hydrogels” can improve the water retention levels of soils [13].

Agricultural applications of chitosan can reduce environmental stress due to drought and soil deficiencies, strengthen seed vitality, improve stand quality, increase yields, and reduce fruit decay of vegetables, fruits and citrus crops [14]. Horticultural application of chitosan increases blooms. The US Forest Service has conducted research on chitosan to control pathogens in pine trees [15, 16] and increase resin pitch outflow which resists pine beetle infestation [17].

The biocontrol mode of action of chitosan elicits natural innate defense responses within plant to resist insects, pathogens, and soil-borne diseases when applied to foliage or the soil [18]. Chitosan increases photosynthesis, promotes and enhances plant growth, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor. When used as seed treatment or seed coating on cotton, corn, seed potatoes, soybeans, sugar beets, tomatoes, wheat and many other seeds, it elicits an innate immunity response in developing roots which destroys parasitic cyst nematodes without harming beneficial nematodes and organisms [19, 20].

The natural biocontrol ability of chitosan should not be confused with the effects of fertilizers or pesticides upon plants or the environment. Chitosan active biopesticides represent a new tier of biological control of crops for agriculture and horticulture [21].

Chitin and chitosan are naturally occurring compounds that display toxicity and inhibit fungal growth and development in agricultural applications. They were reported to be active against

viruses, bacteria and other pests and have been utilized to control disease or reduce their spread, to chelate nutrient and minerals, preventing pathogens from accessing them, or to enhance plant innate defenses. Fragments from chitin and chitosan are known to have eliciting activities leading to a variety of defense responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related proteins and proteinase inhibitors, lignin synthesis, and callose formation. When used to enhance plant defenses, chitin and chitosan induce host defense responses in both monocotyledons and dicotyledons [22].

Soil amendment with chitosan has repeatedly been shown to control fungal diseases in numerous crops, especially *Fusarium* wilts [23–25] and grey mould [26, 27]. It is also of note that these studies show chitosan to be fungistatic against both biotrophic and necrotrophic pathogens.

The control of oomycete pathogens has also been achieved with chitosan treatment, with *Phytophthora capsici* controlled on peppers [28] and *Phytophthora infestans* on potato [29]. This is despite oomycetes lacking chitinous cell walls, like true fungi (eumycota). In the study by Xu *et al.* [28] on *Phytophthora capsici* in peppers, it was reported that the main effect observed in the pathogen was the disruption of the endomembrane system, especially the integrity of the vacuoles.

Foliar application of chitosan has been reported in many systems and for several purposes. Bittelli *et al.* [30] suggested that chitosan might be an effective anti-transpiring to preserve water resources use in agriculture. In their investigation, they examined the potential of foliar applications of chitosan on pepper plants transpiration in the growth room and in the field.

Using scanning electron microscopy and histochemical analyses, stomata were shown to close in response to treatment with chitosan, resulting in a decrease in transpiration. Reduced water use of pepper plants upon treatment with chitosan was estimated at 26–43%, while there was no change in biomass production or yield.

Chitosan has also been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests [31]. It has also been used to increase yield and tuber quality of micro-propagated greenhouse-grown potatoes [32]. Similarly, Faoro *et al.* [33] showed that the use of chitosan applied as a foliar spray on barley reduced locally and systemically the infection by powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*.

While chitosan treatments have been found to be effective at controlling herbivorous insect pests, it is suggested that chitin-based products could potentially be less harmful to non-target insects such as carnivorous species) than conventional insecticides. However, there is not enough published data on other beneficial insects, such as pollinators, to come to firm conclusions on this matter [9].

Chitosan utilized as a soil amendment was shown to control *Fusarium* wilts in many plant species [34]. *Aspergillus flavus* was also completely inhibited in field-grown corn and peanut after soil treatment with chitosan [35]. Part of the effect observed by chitosan on the reduction of soilborne pathogens comes from the fact that it enhances plant defense responses. The other part is linked to the fact that this biopolymer is composed of polysaccharides that stimulate the activity of beneficial microorganisms in the soil such as *Bacillus*, fluorescent *Pseudomonas*, actinomycetes, mycorrhiza and rhizobacteria [36, 37]. This alters the microbial equilibrium in the rhizosphere disadvantaging plant pathogens.

Chitosan is often used in plant disease control as a powerful elicitor rather than a direct antimicrobial or toxic agent. Direct activity of chitosan against viruses has been shown to vary according to molecular weight [38].

Against, bacteria, fungi, oomycetes and other pests, it seems that chitosan is likely to operate indirectly *via* other means such as the enhancement of host resistance. However, a number of studies have shown that chitosan, at defined concentrations, presents antimicrobial properties [39, 40, 41]. For instance, chitosan was reported to exert an inhibitory action on the hyphal growth of numerous pathogenic fungi, including root and necrotrophic pathogens, such as *Fusarium oxysporum*, *Botrytis cinera*, *Monilina laxa*, *Alternaria alternata* and *Pythium aphanidermatum* [42, 43 to 49] besides inhibiting spore germination in some of them [35].

Chitosan is known to possess anti-bacterial properties. These properties also allow for its use as an active ingredient in anti-microbial pesticides. However, as an agricultural active ingredient, Chitosan is best known as a plant growth regulator that boosts the ability of plants to defend against fungal infections. As a plant growth regulator it is applied through foliar application and aids in defending plants against fungal diseases, mold and mildew [50].

There is now a substantial body of evidence that the addition of chitin alters the environmental conditions in the rhizosphere and phyllosphere to shift the microbial balance in favour of beneficial organisms and to the detriment of plant pathogens. Adding chitin-based products to the growing environment may aid beneficial antagonists by stimulating the production and activation of chitinases that can then be used to attack pests and pathogens, or be used as a stable nitrogen-rich polysaccharide food source that boosts the population to the level where other mechanisms control the plant pathogens [9].

References:

- [1] Linden, James C.; Stoner, Richard J.; Knutson, Kenneth W.; Gardner-Hughes, Cecilie A. Organic disease control elicitors. *Agro Food Industry Hi-Tech*. **2000**, 11 (5): 32–4.
- [2] Hadwiger, Lee A. *Multiple effects of chitosan on plant systems: Solid science or hype*. *Plant Science*. **2013**, 208: 42–9. doi:10.1016/j.plantsci.2013.03.007. PMID 23683928.
- [3] Guan, Y.J.; Hu, J.; Wang, X.J.; Shao, C.X. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci. B*. **2009**, 10, 427–433.
- [4] Bhaskara Reddy, M.V.; Arul, J.; Angers, P.; Couture, L. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *J. Agric. Food Chem*. **1999**, 47, 1208–1216.
- [5] Yen, M.T.; Mau, J.L. Selected physical properties of chitin prepared from shiitake stipes. *Food Sci. Technol*. **2007**, 40, 558–563.
- [6] White, R.E. *Principles and Practice of Soil Science: The Soil as a Natural Resource*, 4th ed.; Blackwell: Oxford, UK, **2006**.
- [7] Spiegel, Y.; Kafkafi, U.; Pressman, E. Evaluation of a protein-chitin derivative of crustacean shells as a slow-release nitrogen fertilizer on Chinese cabbage. *J. Hortic. Sci*. **1988**, 63, 621–628.

- [8] Roberts, P.; Jones, D.L. Microbial and plant uptake of free amino sugars in grassland soils. *Soil Biol. Biochem.* **2012**, *49*, 139–149.
- [9] Sharp, Russell G. A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields. *Agronomy* **2013**, *3*, 757-793; doi:10.3390/agronomy3040757.
- [10] Ramírez, M.Á.; Rodríguez, A.T.; Alfonso, L.; Peniche, C. Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotechnol. Apl.* **2010**, *27*, 270–276.
- [11] Bohn, H.L.; Myer, R.A.; O'Connor, G.A. *Soil Chemistry*; John Wiley & Sons: New Jersey, NJ, USA, **2002**.
- [12] Tamura, H.; Nagahama, H.; Tokura, S. Preparation of chitin hydrogel under mild conditions. *Cellulose* **2006**, *13*, 357–364.
- [13] Jamnongkan, T.; Kaewpirom, S. Potassium release kinetics and water retention of controlled-release fertilizers based on chitosan hydrogels. *J. Polym. Environ.* **2010**, *18*, 413–421.
- [14] Linden, J. C.; Stoner, R. J. Pre-harvest application of proprietary elicitor delays fruit senescence. *Advances in Plant Ethylene Research*. **2007** Pp. 301–2. doi:10.1007/978-1-4020-6014-4_65. ISBN 978-1-4020-6013-7.
- [15] Mason, Mary E.; Davis, John M. Defense Response in Slash Pine: Chitosan Treatment Alters the Abundance of Specific mRNAs. *Molecular Plant-Microbe Interactions*. **1997**, *10* (1): 135–7. doi:10.1094/MPMI.1997.10.1.135. PMID 9002276.
- [16] Klepzig, Kier D.; Walkinshaw, Charles H. Cellular response of loblolly pine to wound inoculation with bark beetle-associated fungi and chitosan. **2003** Res. Pap. SRS-30. Asheville, NC U.S. Department of Agriculture, Forest Service, Southern Research Station.
- [17] O'Toole, Erin. Solution for Pine Bark Beetles May Help Front Range Trees. NPR Morning Edition – KUNC 91.5 FM Greeley, CO. **2009**.
- [18] Linden, J.C.; Stoner, R.J. Proprietary Elicitor Affects Seed Germination and Delays Fruit Senescence” (PDF). **2005**. *Journal of Food, Agriculture & Environment*.
- [19] “Smiley R., Cook R.J., Pauliz T. Seed Treatment for Sample Cereal Grains Oregon State University, 2002, EM 8797” (PDF) **2006**.
- [20] “Stoner R., Linden J. Micronutrient elicitor for treating nematodes in field crops, **2006**, Patent Pending, Pub. No.: US 2008/0072494 A1”.
- [21] Goosen, Mattheus F. A. Applications of Chitan and Chitosan. **1996**. CRC Press. Pp. 132–9. ISBN 978-1-56676-449-0.
- [22] Abdelbasset El Hadrami, Lorne R. Adam, Ismail El Hadrami and Fouad Daayf, Chitosan in Plant Protection. *Marine Drugs* **2010**, *8*, 968-987; doi:10.3390/md8040968
- [23] Rabea, E.I.; El Badawy, M.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* **2003**, *4*, 1457–1465.
- [24] Laflamme, P.; Benhamou, N.; Bussi eres, G.; Dessureault, M. Differential effect of chitosan on root rot fungal pathogens in forest nurseries. *Can. J. Bot.* **1999**, *77*, 1460–1468.
- [25] Bell, A.A.; Hubbard, J.C.; Liu, L.; Davis, R.M.; Subbarao, K.V. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows in celery. *Plant Dis.* **1998**, *82*, 322–328.

- [26] Aziz, A.; Trotel-Aziz, P.; Dhuicq, L.; Jeandet, P.; Couderchet, M. Vernet, G. Chitosan oligomers and copper sulphate induce grapevine defense reaction and resistance to grey mould and down mildew. *Phytopathology* **2006**, *96*, 1188–1194.
- [27] Ben-shalom, N.; Ardi, R.; Pinto, R.; Aki, C.; Fallik, E. Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan. *Crop Prot.* **2003**, *22*, 285–290.
- [28] Xu, J.; Zhao, X.; Han, X.; Du, Y. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. *Pestic. Biochem. Physiol.* **2007**, *87*, 220–228.
- [29] O'Herlihy, E.A.; Duffy, E.M.; Cassells, A.C. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folio Geobotanica* **2003**, *38*, 201–208.
- [30] Bittelli, M.; Flury, M.; Campbell, G.S.; Nichols, E.J. Reduction of transpiration through foliar application of chitosan. *Agric. Forest Meteorol.* **2001**, *107*, 167–175.
- [31] Rabea, E.I.; El Badawy, M.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* **2003**, *4*, 1457–1465.
- [32] Kowalski, B.; Jimenez Terry, F.; Herrera, L.; Agramonte Peñalver, D. Application of soluble chitosan *in vitro* and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Res.* **2006**, *49*, 167–176.
- [33] Faoro, F.; Maffi, D.; Cantu, D.; Iriti, M. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. *BioControl* **2008**, *53*, 387–401.
- [34] Rabea, E.I.; El Badawy, M.T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* **2003**, *4*, 1457–1465.
- [35] El Ghaouth, A.; Arul, J.; Asselin, A.; Benhamou, N. Antifungal activity of chitosan on postharvest pathogens: induction of morphological and cytological alterations in *Rhizopus stolonifer*. *Mycol. Res.* **1992**, *96*, 769–779.
- [36] Bell, A.A.; Hubbard, J.C.; Liu, L.; Davis, R.M.; Subbarao, K.V. Effects of chitin and chitosan on the incidence and severity of *Fusarium* yellows in celery. *Plant Dis.* **1998**, *82*, 322–328.
- [37] Murphy, J.G.; Rafferty, S.M.; Cassells, A.C. Stimulation of wild strawberry (*Fragaria vesca*) arbuscular mycorrhizas by addition of shellfish waste to the growth substrate: interaction between mycorrhization, substrate amendment and susceptibility to red core (*Phytophthora fragariae*). *Appl. Soil Ecol.* **2000**, *15*, 153–158.
- [38] Kulikov, S.N.; Chirkov, S.N.; Il'ina, A.V.; Lopatin, S.A.; Varlamov, V.P. Effect of the molecular weight of chitosan on its antiviral activity in plants. *Prik. Biokhim. Mikrobiol.* **2006**, *42* (2), 224–228.
- [39] Sudarshan, N.R.; Hoover, D.G.; Knorr, D. Antibacterial action of chitosan. *Food Biotechnol.* **1992**, *6*, 257–272.
- [40] Kendra, D.F.; Hadwiger L.A. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. *Exp. Mycol.* **1984**, *8*, 276–281.
- [41] Sekiguchi, S.; Miura, Y.; Kaneko, H.; Nishimura, S.I.; Nishi, N.; Iwase, M.; Tokura, S. Molecular weight dependency of antimicrobial activity by chitosan oligomers. In Food

Hydrocolloids: Structures, Properties and Functions; Nishinari, K., Doi, E., Eds.; Plenum: New York, NY, USA, **1994** pp. 71–76.

[42] El Hassni, M.; El Hadrami, A.; Daayf, F.; Chérif, M.; Ait Barka, E.; El Hadrami, I. Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. *Phytopathol. Mediterr.* **2004**, *43*, 195–204.

[43] Leuba, J.L.; Stoessel, P. Chitosan and other polyamines: Anti-fungal activity and interaction with biological membranes. In *Chitin in Nature and Technology*; Muzzarelli, R.A.A., Jeuniaux, C., Gooday, G.W., Eds.; Plenum Press: New York, NY, USA, **1986** pp. 215–222.

[44] Benhamou, N. Ultrastructural detection of β -1,3-glucans in tobacco root tissues infected by *Phytophthora parasitica* var. *nicotianae* using a gold-complexed tobacco β -1,3-glucanase. *Physiol. Mol. Plant Pathol.* **1992**, *41*, 351–357.

[45] Benhamou, N.; Kloepper, J.W.; Tuzun, S. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta* **1998**, *204*, 153–168.

[46] El Ghaouth, A.; Arul, J.; Wilson, C.; Benhamou, N. Ultrastructural and cytochemical aspects of the effect of chitosan on decay of bell pepper fruit. *Physiol. Mol. Plant Pathol.* **1994**, *44*, 417–432.

[47] Romanazzi, G.; Nigro, F.; Ippolito, A.; Di Venere, D.; Salerno, M. Effects of pre-and postharvest chitosan treatments to control storage grey mold of table grapes. *J. Food Sci.* **2002**, *67*, 1862–1867.

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

[49] El Hassni, M. Interaction Palmier dattier-*Fusarium oxysporum albedinis*: Elicitation des réactions de défense et développement de nouvelles stratégies pour le biocontrôle de la maladie du bayoud. PhD Thesis, Faculté des Sciences Semlalia: Marrakech, Morocco, **2005**.

[50] Chitin and Chitosan. Final Registration Review Decision Case 6063. Docket Number EPA-HQ-2007-0566; Chitin Case 6063 www.regulations.gov.