

Chitin and its derivatives as biopolymers with potential agricultural applications

✉ Miguel Á Ramírez¹, Aida T Rodríguez¹, Luis Alfonso², Carlos Peniche³

¹Estación Experimental del Arroz, Instituto Nacional de Ciencias Agrícolas, INCA
Carretera a La Francia Km 1½, CP 22900, Los Palacios, Pinar del Río, Cuba

²Centro Nacional de Investigaciones Científicas, CNIC
Ave. 25 e/ 158 y 190, Cubanacán, CP 10600, Playa, Ciudad de La Habana, Cuba

³Centro de Biomateriales
Ave. Universidad S/N e/ G y Ronda, CP 10400, Ciudad de La Habana, Cuba
E-mail: miguelar@inca.edu.cu

ABSTRACT

Chitin is a biodegradable polymer widely spread in nature. It is mainly obtained from crustacean shells. Chitin and its derivatives have shown to be effective in controlling plagues and plants diseases. Their mechanism of action is strongly linked to their chemical structures. These mechanisms can result from the direct action on the pathogen or can be a consequence of its capacity to induce defensive mechanisms on plants. In any case, the effect is their protection against various vegetable diseases, before and after harvest. The addition of chitin and its derivatives to the soil favours the growth and activity of many chitinolytic organisms that constitute biological controls and are natural enemies of many agents responsible for vegetable plagues and diseases, generating a synergistic effect. On the other side, these biopolymers also favour the growth and development of beneficial microorganisms that establish synergistic relationships with plants, such as as mycorrhizas or *Rhizobium* species. On top of that, increasing the microbial population and activity in the soil improves the properties of nutrients and their availability. As growth regulators, it has been established that these biopolymers accelerate seeds germination, the ability of plants to grow as well as the agricultural yield. It is concluded that chitin and its derivatives have great potential for applications in agriculture. It is foreseen that in the future these biopolymers will be used in greater extension, mainly for substituting actual chemical pesticides or as growth regulators.

Keywords: chitin, agriculture, biopolymer, growth regulator, biological control

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RESUMEN

La quitina y sus derivados, biopolímeros con potencialidades de aplicación agrícola. La quitina es un polímero biodegradable muy abundante en la naturaleza, que se obtiene fundamentalmente del exoesqueleto de los crustáceos, y del que mucho se ha escrito por sus potencialidades de aplicación en la agricultura. Ella y sus derivados son efectivos en el control de enfermedades y plagas vegetales. Sus mecanismos de acción están vinculados a su estructura química. Pueden actuar sobre el organismo patógeno, o inducir mecanismos defensivos en las plantas, contra varias enfermedades vegetales antes y después de la cosecha. La adición de quitina y sus derivados al suelo, favorece el crecimiento y la actividad de muchos organismos quitinolíticos, por un efecto sinérgico. Estos constituyen controles biológicos y enemigos naturales de muchos agentes causales de enfermedades y plagas vegetales. Además, favorecen el crecimiento y desarrollo de microorganismos beneficiosos que establecen relaciones simbióticas con las plantas, tales como las micorrizas o especies del género *Rhizobium*. A su vez, incrementan la población y la actividad microbiana en el suelo, lo que mejora la disposición de nutrientes y sus propiedades. Como reguladores del crecimiento, aceleran la germinación de las semillas, el vigor de las plantas, y el rendimiento agrícola. Por tanto, por su gran potencial de aplicación en la agricultura, se augura que se utilizarán con una mayor extensión, principalmente como sustitutos de los actuales plaguicidas químicos o como reguladores del crecimiento de las plantas.

Palabras clave: quitina, agricultura, biopolímero, regulador del crecimiento, control biológico

Introduction

The use of bioactives compatible with the environment is one of the main challenges for modern agriculture. For this purpose, the use of chitin and its derivatives is a promising alternative, based on its biological activity and easy-to-obtain procedures.

Several studies show the mechanisms of action and the efficiency of such active principles in agriculture, mainly at laboratory scale and under controlled environmental conditions. However, there are few field study reports and low reproducibility of results, espe-

cially studies of scaling up technologies for applying those derivatives at open field production. This has been influenced by dispersion of the available information, and the lack of technical and practical details required to reproduce them, among other aspects. Researches concerning these elements, from Cuba and other countries, are gathered here to facilitate the availability of data for applying chitin and its derivatives in agriculture, and the investigations aimed to introduce such bioproducts in the Cuban agriculture.

✉ Corresponding author

General properties of chitin and its derivatives

Chitin is the second most abundant polysaccharide in nature after cellulose. Chitin bearing a high regeneration rate, with annual estimates of at least 1×10^9 tons being synthesized and degraded every year in nature [1]. This substance is found in cellular structures of fungi [2], bacteria [3], insects [4], arachnids [5], crustaceans [6], nematodes [7] and other invertebrates such as: annelids, mollusks, cephalopods and hemichordates [8].

Chitin is a white, partially crystalline, odorless and tasteless solid at its pure state. It is made of N-acetyl-2-amino-2-desoxy-D-glucose aminosaccharide units, linked together by $\beta(1 \rightarrow 4)$ glycosidic bonds to form a linear chain, some of the residues appearing deacetylated [9]. Therefore, chitin shows a structure that resembles cellulose, except for the carbon residue at position 2 which has an acetamide group attached to chitin instead of the hydroxyl group of cellulose (Figure 1).

Other relevant properties of this polymeric bi-product are its high molecular weight, and its porous structure favoring high water absorption [10].

Its properties as a product vary depending on the source from which it was obtained and prepared. This has led to further developments to improve production methods and to achieve more convenient properties for different uses [11].

As Chitin is insoluble in water, a characteristic that limits its application, working with some of its direct derivatives will be more convenient than with the natural polymer. Chitosan is the most relevant derivative, and it can be found in nature or can be obtained in synthetic form (Figure 1), composed mainly by deacetylated units, influencing its chemical and biological properties. Chitosan is soluble in diluted acid solutions, and is also among the few cationic polymers found in nature, with amino groups able to get positive charges and responsible in part for its potent antimicrobial activity [12, 13].

The high viscosity of chitosan solutions is also a relevant characteristic that favors its biological properties, which are determined in general by a number of factors, including the average molecular weight of the polymer, acetylation degree and solution concentration, among others. Films and threads for dressing can be obtained from chitosan solutions for a great number of industrial applications [14].

Other chitin derivatives are oligosaccharides of 2 to 20 N-acetylglucosamine residues in length. Their lower molecular weights provide them with chemical and biological properties other than those of the original polymer, such as water solubility and signaling functions during symbiotic interactions in plants [15].

Chitin and all its derivatives share a high nitrogen content (6.14-8.3%) and high thermal and chemical stability [2]. Nevertheless, they are also substrates very susceptible to degradation by several enzyme families, this aspect derived from their composition and natural origin [16]. The presence of functional hydroxyl and amino groups (these in the deacetylated units) support the formation of coordination compounds (complexes) with metal ions of copper, zinc

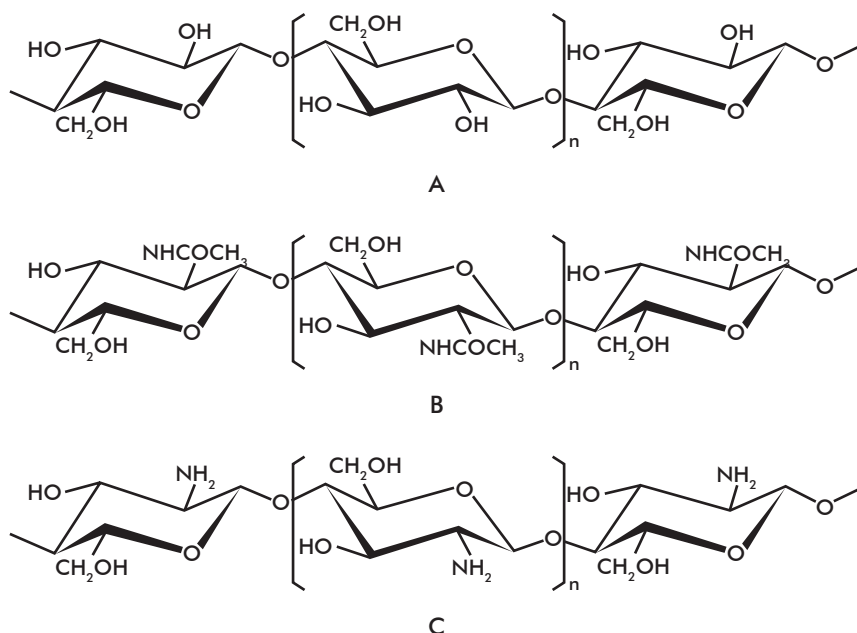


Figure 1. Structural representation A) cellulose, B) fully acetylated chitin and C) fully deacetylated chitosan, evidencing their structural similarity.

and iron and others, but not with those of alkaline (e.g., sodium or potassium) or alkaline earth (e.g., calcium or magnesium) metals. These complexes show a strong antimicrobial activity against some vegetable pathogens, being promising agents for agricultural application [17]. Moreover, they bear absorptive properties, very useful to remove stains [18, 19], residuals from water [20], and they are useful for other applications. All these make chitin and their derivatives highly applicable to human activity [21].

Preparation of chitin and its derivatives

Chitin can be obtained from several sources, but mainly from crustacean and fungi debris. Of them, fungi materials are hard to be produced at high scale for marketing [22]. Therefore, the source of choice is the crustacean processing waste, due to its abundance, chitin content, and also to ameliorate its high contaminant effect [23].

It has been estimated that over 170 000 tons out of the 1 440 000 tons of the chitinous wastes globally obtained per year come from the global fish industry, all of them accounting for an estimate of more than 25 000 tons of chitin if processed [24].

Several methods have been established to produce chitin from natural sources, essentially involving acidic treatment for materials' demineralization and with alkali for protein separation. Pigments and fat can be optionally removed. Nevertheless, some of these components make the resulting chitin very useful for certain applications, especially for agriculture. Therefore, the product's purity grade is defined by its final application [25].

In Cuba, over 8000 tons of lobster are captured every year, 30% corresponds to about 1500 tons which are discarded and used as raw material for chitin production [26]. Wastes coming from other marketable species as shrimp, and sea and freshwater

1. Gooday GW. The ecology of chitin degradation. *Adv Micro Ecol* 1990;11:387-419.
2. Yen MT, Mau JL. Selected physical properties of chitin prepared from shiitake stipes. *Food Sci Technol* 2007;40(3): 558-63.
3. Gomes RC, Soares RMA, Nakamura CV, Souto-Padrón T, de Souza RF, de Azevedo Soares Semêdo LT, et al. *Streptomyces lunalinharesii* spores contain chitin on the outer sheath. *FEMS Microbiol Lett* 2008;286(1):118-23.
4. Majtán J, Bíliková K, Markovic O, Gróf J, Kogan G, Simúth J. Isolation and characterization of chitin from bumblebee (*Bombus terrestris*). *Int J Biol Macromol* 2007;40(3):237-41.
5. Wei-Bing, Shi-Ming, Feng. Lethal effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with a description of a mite egg bioassay system. *Biol Control* 2004;30:165-73.
6. Díaz-Rojas EI, Argüelles-Monal WM, Higuera-Ciapara I, Hernández J, Lizardi-Mendoza J, Goycoolea FM. Determination of chitin and protein contents during the isolation of chitin from shrimp waste. *Macromol Biosci* 2006;6(5):340-47.
7. Fanellia E, Vitob MD, Jonesc JT, Giorgia CD. Analysis of chitin synthase function in a plant parasitic nematode, *Meloidogyne artiellia*, using RNAi. *Gene* 2005;349: 87-95.
8. Wu SD, Wu C-S, Chen H. Cuticle structure of squid *Illex argentinus* pen. *Fish Sci* 2003;69:849-55.
9. Peniche C. Estudios sobre quitina y quitosana. [Tesis en opción del título de Doctor en Ciencias]. Editorial Universitaria, Universidad de La Habana, 2006.

crabs are also used. In fact, several procedures were developed to prepare chitin depending on the application. For example, the method that generates high quality and pure chitin for pharmaceutical application is regarded as one of the first among Ibero-American countries [27]. At present there are two factories in Cuba producing this pharmaceutical-grade chitin at levels overreaching the demands.

Additionally, there is a process for integral processing of the wastes [28], and another designed to prepare chitin and its derivatives for specific application in agriculture [29].

Biological activity of chitin in agriculture

Chitin and its derivatives are biologically active during its interaction with plants and microorganisms [30]. Four main approaches have been identified for chitin application in agriculture (see table 1):

1. Protection of plants from pests and diseases before and after harvest.
2. Enhancing of antagonist microorganisms action and biological controls.
3. Enhancing the beneficial symbiotic plant-microorganism interactions; and
4. Regulating plant growth and development.

Some results evidenced that polymeric chitin does not show a substantial antimicrobial activity affecting growth and development of plant pathogens. This is determined by its insolubility in water and compact structure. Otherwise, its deacetylated derivative chitosan has certainly shown a potent antimicrobial activity, due to protonation of its amino groups in solution. These results are in agreement with those obtained for soluble chitin and chitosan oligomers at the National Institute of Agricultural Sciences (INCA, Cuba). Moreover, positively charged oligochitosans showed antifungal activity, which was absent in chitin oligomers (uncharged) when compared to the control treatment [31].

Vegetal membranes respond to polymeric chitin and its derivatives by surmounting a cascade of enzymatic reactions which ultimately results in induced and systemic resistance in plants [32]. This has been corroborated by isolating chitin-specific membrane receptors in soybean and rice [33].

Chitin has also been used to enhance the efficiency of natural biological controls. Many microorganisms acting as antagonists use chitinases against plant pests and diseases (e.g., *Trichoderma* sp.). These enzymes are overproduced together with other hydrolases in the presence of chitins and some of its derivatives, increasing the efficiency of microorganisms acting as biocontrols [34].

Recent findings demonstrate that chitin and its derivatives can improve legume-*Rhizobium* symbiosis. Nodulation factors excreted by *Rhizobium* sp. are 3-to-5-units chitin fragments bound to fatty acid and protein ramifications [35]. Therefore, chitin can be provided as precursor substrate for these metabolites. Other types of interactions (e.g., mycorrhization) have benefited from adding chitin derivatives, as in tomato cultivation [36].

Moreover, chitin was demonstrated to favor plant growth and development by increasing enzyme and

Table 1. Some uses of chitin and its derivatives in agriculture

Use	Crop	Properties	Compound	Reference
Protection after harvest	Mango	Antimicrobial	Chitosan	[37-39]
	Guava	Antimicrobial		
	Tomato	Antimicrobial		
Retardation of fruit ripening process	Papaya	Semipermeable film formation	Chitosan	[40]
Defensive enzymes stimulation	Rice	Inducer	Chitin	[41-43]
	Tobacco		Chitosan	
	Pea		Chitosan	
Mycorrhizal symbiosis stimulator	Tomato	Inducer of recognition mechanisms	Chitin	[44]
Nematocidal control	Tomato	Increases soil chitinolytic microbiotes	Chitin	[45]
Biocontrol action enhancer	Peanut	Stimulator substrate for hydrolases enzymes	Chitin	[46]
	Apple			

metabolic functions, also accelerating germination [30].

Crop protection from pests and diseases

Chitin and its derivatives have been used to protect crops from diseases either before or after harvest, directly or indirectly, depending on the specific plant-pathogen interaction. Some results of such applications are shown in the following by pathogen group.

Antifungal activity

Plants are protected from fungi by the biological activity of chitin and its derivatives through two main mechanisms: i) direct antifungal action of these molecules, affecting fungal growth and development; and ii) activation of defensive mechanisms interfering or inhibiting pathogen's development, subsequently halting or limiting disease progression.

Regarding chitin derivatives, especially those bearing highly reactive functional groups as chitosan and derived compounds, they were demonstrated as having direct antifungal activity on phytopathogenic fungi [47]. This is influenced by the compound's chemical properties and concentration. In this sense, chitosan polymers administered at 1 g/L completely inhibited *Rhizoctonia solana* mycelial growth, such inhibition being limited to 80% at a 500 mg/L concentration [32]. The inhibition was further reduced to 50% by decreasing the polymer molecular weight by hydrolysis, once the hydrolyzate at 500 mg/L was applied. A minimal 20% of inhibition was obtained by increasing chitin acetylation degree and delivering it as colloid. Several studies confirmed these results, highlighting the relevance of fungal type [48]. *In vitro* inhibition of mycelial growth fluctuated in a sample of 14 different phytopathogenic fungi, depending on acetylation degree and molecular weight of the chitin derivatives assayed. Nevertheless, a tendency towards growth inhibition was observed by increasing both deacetylation and molecular weight of the compounds tested.

Regarding the mechanisms of action of chitin and its derivatives, it was established that free amino group protonation on a slightly acidic medium enhanced antifungal activity. Some authors point out that the positively charged compounds interact better at chromosome level, improving the expression of ge-

10. Tamura H, Nagahama H, Tokura S. Preparation of chitin hydrogel under mild conditions. *Cellulose* 2006;13(4):357-64.

11. Rinaudo M. Chitin and chitosan: Properties and applications. *Prog Polym Sci* 2006;31(7):603-32.

12. Harish Prashanth KV, Tharanathan RN. Chitin/chitosan: modifications and their unlimited application potential -an overview. *Trends Food Sci Technol* 2007;18(3):117-31.

13. Xu J, Zhao X, Han X, Du Y. Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. *Pest Biochem Physiol* 2007;87(3):220-8.

14. Pillai CKS, Paul W, Sharma CP. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog Polym Sci* 2009;34(7):641-78.

15. Gil-Serrano AM, Franco-Rodríguez G, Tejero-Mateo P, Thomas-Oates J, Spaink HP, Ruiz-Sainz J, et al. Structural determination of the lipo-chitin oligosaccharides nodulation signals produced by *Rhizobium fredii* HH103. *Carbohydr Res* 1997;303:435-43.

16. Li J, Du Y, Liang H. Influence of molecular parameters on the degradation of chitosan by a commercial enzyme. *Polymer Degrad Stabil* 2007;92(3):515-24.

17. Cárdenas R, Ramírez M. Efecto de los derivados de quitina y su combinación con sulfato de cobre en el comportamiento del crecimiento micelial y esporulación de un aislamiento monospórico del hongo *Pyricularia grisea*, Sacc. *Cultiv Trop* 2004; 25(4):89-93.

18. Klimiuk E, Gusiatis Z, Kabardo K. The effectiveness of surfactants adsorption on chitin and dye-modified chitin. *Polish J Environ Studies* 2006;15(1):95-104.

19. Crini G, Badot PM. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes. *Prog Polym Sci* 2008;33(4):399-447.

20. Crini G. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Prog Polym Sci* 2005;30(1):38-70.

21. Rudrapatnam N. Chitin - The undisputed biomolecule of great potential. *Crit Rev Food Sci Nutr* 2003;43(1):61-87.

nes involved in plant resistance [43]. It was also proposed that the action occurred in an indirect manner by making Ca^{2+} inaccessible, essential nutrients and minerals for the growth of filamentous fungi. There are other reports on their interaction with the plasma membrane, interfering with its functions as chelating agents and modifying membrane permeability [49]. It is also suggested that the activity affects *Rhizopus stolonifer* in influencing the balance between biosynthesis and degradation of cell wall components.

Results obtained at INCA's laboratories indicate that chitosan and its oligomers completely inhibit *Pyricularia grisea* mycelial growth at 1 g/L and pH 5.0 in the culture medium [50]. Noteworthy, the pH of the resulting solution affects the positive charge of amino groups, since fungi growth was just slightly inhibited at pH 6, while sporulation remained fully arrested [17].

Similarly, the presence of chitosan at 1000 mg / L affected the growth of *Sarocladium oryzae* by more than 40% compared to control [51]. Some studies have remarked the relevance of fungi family and genus on its susceptibility to chitin derivatives, with cell wall chitin content as a factor that explains the differences observed. Mycelial growth of some species as *Phytophthora parasitica* are inhibited at low chitosan concentrations (i.e., 100 mg/L), while other require higher concentrations for significant inhibition (as *Fusarium oxysporum radici licopersicii*, over 1 g/L).

Particularly, the artificial stimulation of plant defensive mechanisms have been studied by adding chitin derivatives as elicitors (elicitation), since they become generally protected by systemic resistance from several diseases [52].

Results from investigations in rice demonstrated that seeds recovered with chitin derivatives increase hydrolytic enzymes production, such as chitinases and β 1,3 glycanases which degrade chitin and 1,3 glycans, respectively. As we know, these two types of compounds are major cell wall components in most phytopathogenic fungi [53, 54].

Besides, chitosan and its positively charged derivatives stimulate plants to produce antifungal metabolites [55]. In spite of these evidences, there are reports on derivatives lacking positive charge, and even insoluble polymeric chitin, as inducing high levels of very potent antimicrobial metabolites (e.g., phytoalexins). Among these molecules are momilactones and oryzaalexins, which completely inhibit *Pyricularia grisea* Sacc at concentrations as low as 0.9 mg/L or even at nanogram scale [32].

Other authors suggest that oligomers could be more effective in plants, because of its smaller molecular size which determines an easier root absorption, or by foliar aspersion [42]. In spite of the advantages of oligomers over the natural polymer, these criteria is not absolute, as demonstrated by other works showing the additional influence of crop type, pathogen's properties (particularly cell wall composition), chitosan acetylation degree and solution pH [56].

Several crops have been evaluated for protection from diseases caused by soil fungal pathogens by delivering chitin derivatives. For example, chitosan protects pepper from *Phytophthora aphanidermatum* [57]. In tomato, partially acetylated chitosans delivered either

by seed or foliar routes induced hydrolytic enzyme production and reduced *Fusarium oxysporum licopersicii*-caused lesions [55]. Other authors found a lower incidence of diseases in wheat and rice, leading to significantly increased production yields [58]. Protection was also detected in peanut, as well as defensive mechanisms [59].

Noteworthy, most of these investigations used chitosan and its derivatives seeking for protection, but it was clearly established that acetylation is essential to induce production of hydrogen peroxide and other oxygen reactive species. These metabolites are the key components triggering enzymatic reaction pathways which ultimately lead to systemic resistance in plants [60].

Although uncharged, chitin and its fragments are potentially protective in plants, mostly in monocotyledonous. Chitin but not chitosan oligomers promote several defensive reactions in rice, wheat, arabidopsis, water melon, bean, soybean and peanut [34, 58, 61, 62]. These responses include the accumulation of pathogenesis-related proteins (e.g., phenylammonium lyase (PAL) and β 1,3 glycanase, chitinase and peroxidases [41]), synthesis of protease inhibitors and phytoalexins [63], lignification [64], callose synthesis and hypersensitive cell death reaction [65].

Antiviral activity

Chitin derivatives display antiviral activity, specially the cationic ones which are very potent at inhibiting locally-produced virus injuries. Its action is attributed to virus infection dependence on surface charge [66]. Nonetheless, neither acetylation nor molecular weight correlates with antiviral activity, since acetylated chitin oligomers inhibited the mosaic alfalfa virus in bean at 0.01%. Additionally, the antiviral activity varies among plant species. Evidences point towards two mechanisms: i) interference with virus adhesion to leave surface; and ii) systemic transmission of resistance to other plant organs, mediated by different enzymes as peroxidase [67, 68].

Noteworthy, these compounds protect plants not only from mechanically but also from vector-borne diseases [56].

Remarkably, 1% chitosan sprays were able to protect tomato from viroids, a very destructive and hard-to-treat plant pathogen [69].

All these results are very valuable for agriculture, due to the almost absolute lack of chemicals able to control plant viral infections.

Anti-bacterial activity in plants

Chitin derivatives can also protect plants from bacterial diseases. *In vitro* studies demonstrated that chitosan and chitin cationic derivatives inhibit growth of 11 different bacteria at concentrations ranging 0.008 to 0.25%, with direct inhibition mainly depending on bacterial type and the derivative used [70]. Other reports showed that cationic derivatives inhibit growth of either gram-positive or -negative bacteria, while the anionic ones require 15-fold concentrations for similar effects [71]. These authors also detected an inverse correlation between chitosan molecular weight and growth inhibition, in agreement with other reports regarding anti-bacterial activity as dependent on the

22. Andrade V, Neto B, Fukushima K, Campos-Takaki G. Effect of medium components and time of cultivation on chitin production by *Mucor circinelloides* (*Mucor javanicus*) IFO 4570[A factorial study. *Rev Iberoam Micol* 2003;20:149-53.

23. Cauchie M. Chitin production by arthropods in the hydrosphere. *Hydrobiologia* 2002;470(1-3):63-95.

24. Goycolea F, Agulló E, Mato R. Fuentes y procesos de obtención. In: Abram APd, editor. *Quitina y Quitosano. Obtención, caracterización y aplicaciones*, Lima, Perú. Pontificia Universidad Católica del Perú, 2004, p.106-54.

25. No H, Meyer S. Preparation and characterisation of chitin and chitosan a review. *J Aquat Food Prod Technol* 1995; 14:27-52.

26. León ME, de Puga R, Baisre J. National report on the lobster fisheries in Cuba. *FAO Fish Rep* 2001;619:197-202.

27. Henriques R, Nieto O, inventors; Instituto de Química y Biología Experimental, assignee. Método para obtención de quitina suficientemente pura. *CU Patent* 20760. 1980.

28. García D, Oviedo C, Nieto JM, Peniche C, Henriquez RD, inventors; Instituto de Química y Biología Experimental, assignee. Método para el aprovechamiento del desecho de la langosta común. *CU Patent* 21658 A1. 1996 Oct 5.

29. Ramírez MA, Cabrera G, Gutiérrez A, Rodríguez T. Metodología para la obtención de quitosana a bajas temperaturas. *Cultiv Trop* 2000;21(1):79-82.

30. Hirano S. Applications of chitin and chitosan in the ecological and environmental fields. In: Goosen MFA, Ed., *Technomic*, Lancaster, PA, 1997.31-54.

31. Parra Y, Ramírez MA. Efecto de diferentes derivados de quitina sobre el crecimiento in vitro del hongo *Rhizoctonia solani* Kuhn. *Cultiv Trop* 2002;23(2):73-5.

32. Ruen YY, Ch W. Elicitation of rice diterpenes phytoalexins by chitin. *Arch Biochem Biophys* 1993;294(3):450-5.

33. Day RB, Okada M, Ito Y, Tsukada K, Zaghouani H, Shibuya N, et al. Biding site for chitin oligosaccharides in the soybean plasma membrane. *Plant Physiol* 2001; 26:1162-73.

34. Kishore GK, Pande S, Podile AR. Chitin-supplemented foliar application of *Serratia marcescens* GPS 5 improves control of late leaf spot disease of Groundnut by activating defence-related enzymes. *J Phytopathol* 2005;153:169-73.

35. Staehelin C, Schultze M, Tokuyasu K, Poinso V, Promé JC, Kondorosi E, et al. N-deacetylation of *Sinorhizobium meliloti* Nod factors increases their stability in the *Medicago sativa* rhizosphere and decreases their biological activity. *Mol Plant Microbe Interact* 2000;13(1):72-79.

36. Iglesias R, Pombo R, Cabrera G, Fernández F, Morales. D. Efecto de la quitina y sus derivados sobre la infección micorrizica y el crecimiento y desarrollo de las plantas de tomate (*Lycopersicon esculentum* Mill). *Cultiv Trop* 1995;16(3):43-4.

37. Chien P-J, Sheu F, Yang F-H. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *J Food Eng* 2007;78(1):225-9.

assayed bacterial type and the molecular weight of the compounds [72].

Moreover, the efficiency of chitosan application to inhibit bacterial infection in tomato was demonstrated as depending on chitosan concentration (inhibition achieved at 0.1%) and timing prior to infection [73].

In other experiments using cationic and anionic chitosan derivatives sprayed on tomato leaves, *Pseudomonas syringae* pv. infection was inhibited 60 to 70% with cationic, while the anionic ones were irrelevant for disease progression. Since some of the cationic derivatives did not inhibit bacterial growth *in vitro* but did *in vivo*, it was suggested that they bear a dual effect, both inhibiting bacterial growth and inducing natural defensive mechanisms in plants [56, 74].

Nematicidal activity

Nematodes have been effectively controlled 0,008 by applying chitin and chitin-like compounds to soils [75]. Once added, chitinolytic microorganisms tend to proliferate, destroying nematode eggs and degrading the chitin-containing cuticle of young nematodes [76]. Moreover, chitin material increase ammonia emissions upon mineralization, at concentrations toxic enough for nematodes, decreasing its population and subsequently reducing their damage to plant roots [77]. This last effect was corroborated by adding nitrification inhibitors, which protect soil-added chitin from degradation and further decrease nematode-mediated damage [78]. As previously mentioned, the increased chitinolytic microbial population resulted in high nematicidal activity in soil, reducing nematode injury in tomato plants [45].

Nematode mortality is remarkably higher for chitin than chitosan, this last of higher nitrogen content, suggesting that the effect of increased populations of nematophagous and nematicidal microorganisms prevail over that of ammonia at toxic levels. [79].

This led to designing enhanced chitin derivatives as the chitin-protein complex, taking advantage of both nematicidal mechanisms. Results showed an effective gradient for nematicidal activity of chitin-protein complex > pure chitin >> chitosan when assayed against nematodes of the *Heterotera* genus [79].

Post-harvest protection of crops

Chitin derivatives, and particularly chitosan, protect fruits from post-harvest diseases, being used as soluble additives to provide anti-microbial properties and capable of forming gas semi-permeable films [30].

Soft rot damage is significantly reduced in tomato by coating with chitosan films [39]. Pre-harvest treatment in strawberry decreases infection levels and improve fruit quality [80, 81]. In carrots, chitosan application three days prior to *Sclerotinia sclerotium* inoculation decreases pathogen incidence, resulting in smaller lesions [82]. Studies in chitosan-treated pepper at storage conditions showed that the gray mold appeared seven days after than in untreated fruits [83]. Chitin derivatives were not only used to coat fruits but also to increase quality of sliced fruits as shown in studies of sliced red pitayas [37] and mango [37, 84]. In general, chitosan has shown a behaviour similar to that of chemical fungicides, so it can be used instead of them, with the advantage of being a biodegradable

product [85]. The use of these alternatives in agriculture is due to the lower production costs of chitin derivatives and its advantages over the currently applied phytosanitary products.

Enhanced biopesticides

Many antagonist organisms and natural biological controls exert their biological activity through chitinase and hydrolase enzymes secretion [86]. Chitin and its derivatives certainly increase their production by microorganisms such as *Tricoderma* sp. and *Bacillus* sp., enhancing its efficiency to control pathogenic microorganisms and pests [87]. For instance, the control of the disease was better achieved by applying a bacterium together with the polymeric chitin in peanut than the one obtained with the microorganism alone. [34]. A better control in *Phytophthora fragaria* was also obtained with the application of chitin although, the time of exposure to chitin is relevant for the control attained [88].

In fact, native populations of biocontrol microorganisms become increased by adding chitin in soils infected with pathogenic agents. Thereafter, these endogenous control strains can be isolated, cultured and potentially used as biological controls, as demonstrated against actinomycetes in sandy soils [89].

Other authors have also demonstrated a significant increase in chitinolytic microorganisms even in very infertile soils like in dunes, improving soil microbiota and its properties [90].

Indeed, chitinases are enzymes relevant for biopesticide control mechanisms, being the hydrolysis of chitin-containing media a common practice to evaluate the efficiency of bioinsecticide organisms.

It has been considered to add chitin derivatives to formulations containing these microorganisms to increase biopesticide effectiveness, to provide a favorable developmental environment and resistance against adverse conditions [46]. All these actions can contribute to improve the use of biological controls in agriculture.

Plant nutrition and soil fertility

Chitin and its derivatives show additional properties among carbohydrates, as nitrogen content and, therefore, a low C/N ratio [1]. This characteristic supports soil microorganism proliferation, especially of those bearing chitinolytic and proteolytic metabolism as actinomycetes. In fact, half the chitin added to the soil becomes mineralized in less than four weeks, a result closely related to soil pH, humidity and organic material [90]. Its addition increases both prokaryote and eukaryote microbial populations and their activities, since they are altogether involved in chitin mineralization, including populations of nitrogen fixation microorganisms, and methane, carbon dioxide and dinitrogen monoxide emissions are raised [91, 92]. Many of these chitinolytic organisms establish beneficial symbiotic interactions with plants, as mycorrhiza and *Rhizobium* spp., favoring vegetal absorption of certain nutrients and especially nitrogen fixation. For example, amendments of chitin together with fertilizers as urea have been used to improve soil microbiota, to control pathogenic organisms and to strengthen plant nutrition, all these showing better results

38. Thommohaway C, Kanlayanarat S, Uthairatanakij A, Jitareerat P. Quality of fresh-cut guava (*Psidium Guajava* L.) as affected by chitosan treatment. *Acta Horticult* 2007;746:449-55.

39. Bautista-Baños S. Evaluación del quitosano en el desarrollo de la pudrición blanda del tomate durante el almacenamiento. *Rev Iber Tecnología Postcosecha* 2004;1:63-67.

40. Alimuniar A, Zainuddin R. An economical technique for producing chitosan. *Proceedings from the 6th International Conference on Chitosan, Poland, 16-19 August, 1994.*

41. Rodríguez AT, Ramírez MA, Cárdenas RM, Hernández AN, Velázquez MG, Bautista S. Induction of defense response of *Oryza sativa* L. against *Pyricularia grisea* (Cooke) Sacc by treating seeds with chitosan and hydrolyzed chitosan. *Pest Biochem Physiol* 2007;89:206-15.

42. Falcón AB, Ramírez MA, Márquez R, Hernández M. Chitosan and its hydrolysate at tobacco-phytophthora parasitica interaction. *Cultiv Tropic* 2002;23(1):61-6.

43. Hadwiger LA, Ogawa T, Kuyama H. Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. *Mol Plant Microbe Interact* 1994;7(4):531-3.

44. Iglesias R, Gutiérrez A, Fernández F. The influence of chitin from lobster exoskeleton on seedling growth and mycorrhizal infection in tomato crop (*Lycopersicon esculentum* Mill). *Cultiv Tropic* 1994; 15(2):48-9.

45. Jin RD, Suh J, Park RD, Kim YW, Krishnan HB, Kil Yong K. Effect of chitin compost and broth on biological control of *Meloidogyne incognita* on tomato (*Lycopersicon esculentum* Mill). *Nematology* 2005;7(1):125-32.

46. Backman P, Rodriguez-Kabana R, Kokalis N, inventors; Auburn University, assignee. Method of controlling foliar microorganism populations. US Patent 5,288,488, 1994 Feb 22.

47. Rabea E, Badawy MT, Stevens CV, Smaghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules* 2003; 4(6):1457-65.

48. Hirano S, Nagao YN. Effect of chitosan, pectic acid on pathogenic fungi. *Agric Biol Chem* 1989;58(1):24-6.

49. Roller S, Covill N. The antifungal properties of chitosan in laboratory media and apple juice. *Int J Food Microbiol* 1999; 47:67-77.

50. Rodríguez AT, Ramírez MA, Nápoles MC, Cárdenas RM. Antifungal activity of chitosan and one its hydrolysates on *Pyricularia grisea* Sacc fungus. *Cultiv Tropic* 2003;24(2):85-8.

51. Cruz A, Rivero D, Martínez B, Ramírez MA, Rodríguez AT. Efecto de la quitosana sobre el crecimiento y desarrollo *in vitro* de *Sarocladium oryzae* Sawada. *Rev Protección Veg* 2004;19(2):133-6.

52. Yamaguchi T, Ito Y, Shibuya N. Oligosaccharide elicitors and their receptors for plant defense responses. *Trends Glycosci Glycotechnol* 2000;12(64):113-20.

than the controls in tomato, carnation and grazing [76, 77, 88].

Biofertilization enhancement

As previously suggested, chitin has been used to enhance beneficial plant-microorganism interactions as mycorrhization, increasing it up to 20% resulting in a significant increase of the performance. [74, 75]. This result was used to generate Ecomic, a Cuban mycorrhiza-based biofertilizer. [93]. It is suggested that the addition of chitin could accelerate the first step in the establishment of mycorrhizal infection, which involves breaking the fungal cell wall by plant chitinases.

On the other hand, and as mentioned above, nodulation factors essential to establish a productive symbiotic legume-*Rhizobium* interaction are partially composed of chitin oligomers [15]. Certainly, N-acetylation of these molecules is a precondition to display biological activity, while structural differences among these compounds serve as specie-specific signature determining the *Rhizobium* to legume association [35]. Further research must address this theoretical knowledge to avoid divergences when extrapolating *in vitro* laboratory results obtained under semi-controlled conditions to field applications.

Regulation of vegetal growth and development

Favorable changes are induced by chitin derivatives in plants and fruits metabolism. For example, chitosan-treated tomato seeds show accelerated germination and produced highly vigorous seedlings [94]. This effect was also observed in cereals [95], and specifically in wheat and rice, where yields were increased at field conditions, these results are being currently scaled up to marketable levels [58].

Our researches revealed that the chemical nature of chitin can significantly influence vegetal growth [96]. Colloidal chitin, a degraded variant of the polymer, accelerated seedling growth in tomato during the

first 15 days as compared to the much more slowly-degraded chitin-protein complex (remaining attached to proteins). Nevertheless, plants treated with this last compound were more vigorous and taller 30 days after treatment. Either the case, plants treated with chitin derivatives showed a faster development than the untreated ones. Soybean seeds coated with depolymerized chitin increased harvest yields in 118% compared to the control [61], and a relatively similar behavior was observed in carrot. These effects of chitin derivatives on vegetal growth led some groups to consider chitin as an exogenous oligosaccharin modulating the physiological response on these crops.

Conclusions

The ubiquity, biological and biocompatible properties of chitin and its derivatives settle them up as promising alternatives for agriculture. Further research is required for laboratory results obtained under controlled conditions in Cuba and other countries to become agricultural practice. Its antiviral activity, together with the rest of recently-discovered properties are highly demanded in agriculture, while others, more established and still underestimated characteristics (e.g., antifungal and nematocidal activities) could result in great steps towards sustainable agricultural practices, by decreasing the use of chemical synthetic pesticides and bringing a new focus to modern phytopathology. Symbiotic interactions of these compounds could readily impact on agriculture production yields. They could be significantly useful under adverse conditions as in low fertility, high salinity and heavy metal-contaminated soils, as in those affected by prolonged drought because of climatic changes. Of course, all these depend on gaining the focus of researchers, farmers and producers on these compounds potentialities. In this sense, future developments for delivering chitin and its derivatives at field scale will irremissibly be among the new challenges to overcome.

53. Hirano S, Yamamoto T, Hayashi M, Nishida T, Inui H. Chitinase activity in seed coated with chitosan derivatives. *J Agric Biol Chem* 1990;54(10):2719-20.

54. Rodríguez AT, Ramírez MA, Falcón A, Guridi F, Cristo E. Estimulación de algunas enzimas relacionadas con la defensa en plantas de arroz (*Oryza sativa*, L.) obtenidas de semillas tratadas con quitosana. *Cultiv Trop* 2004;25(3):111-5.

55. Benhamou N, Lafontaine P, Nicole JN. Induction of systemic resistance to *Fusarium* crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathology* 1994;84(12):1432-44.

56. Potential use of chitosan in plant protection. In: *Chitin and chitosan*. Polish-Russian Monograph. Eds: Struszczyk H, Pospieszny H and Gamzazade A, 1999, p. 115-130.

57. El Ghaouth A, Arul J, Grenier J, Benhamou N, Asselin A, Belanger G. Chitosan induces systemic resistance against *Phytophthora blight* in greenhouse grown cucumber. *Phytopathology* 1994; 84(9):1120-7.

58. Hadwiger LA, inventor; Washington State University Research Foundation (Pullman, WA), assignee. Method for treating cereal crop seed with chitosan to enhance yield root growth, and stem strength. US patent 5,104,437. 1992 Apr 14.

59. Sathibayama M, Balasubramanian R. Chitosan induces resistance components in arachi hipogaea against leaf rust caused by *Puccinia arachidis*. *Crop Prot* 1998; 17:307-13.

60. Kauss H, Jeblick W, Domard A, Siegrist J. Partial acetylation of chitosan and a conditioning period are essential for elicitation of H₂O₂ in surface-abraded tissues from various plants. *Adv Chitin Sci* 1997;2:94-101.

61. Hirano S, Hayashi M, Okuno S. Soybean seeds surface-coated with depolymerised chitins: chitinase activity as a predictive index for the harvest of beans in field culture. *J Sci Food Agric* 2000;81(2):205-9.

62. Wan J, Shuqun Zhang, Stacey G. Activation of a mitogen-activated protein kinase pathway in *Arabidopsis* by chitin. *Mol Plant Pathol* 2004;5(2):125-35.

63. Nahalka J, Nahalkov J, Gemeiner P, Blan P. Elicitation of plumbagin by chitin and its release into the medium in *Drosophyllum lusitanicum* Link. suspension cultures. *Biotechnol Lett* 1998;20(9):841-5.

64. Maksimov IV, Cherepanova EA, Khairullin RM. Chitin specific-peroxidases in plants. *Biochemistry (Moscow)* 2003; 68(1):111-5.

65. Prapagdee B, Kotchadat K, Kumsopa A, Visarathanonth N. The role of chitosan in protection of soybean from sudden death syn-

drome caused by *Fusarium solani* f. sp. *glycines*. *Bioresour Technol* 2007; 98(7):1353-8.

66. Pospieszny H, Chirkov S, Atabekov L. Induction of antiviral resistance in plant by Chitosan. *Plant Sci* 1991;79:63-9.

67. Pospieszny H, Giebel J. Peroxidase activity is related to th resistance against viruses induced by chitosan. *Chitin Enzymol* 1996; 2:379-83.

68. Iriti M, Sironi M, Gomarasca S, Casazza AP, Soave C, Faoro F. Cell death-mediated antiviral effect of chitosan in tobacco. *Plant Physiol Biochem* 2006;44(11-12): 893-900.

69. Pospieszny H. Antiviral activity of chitosan. *Crop Prot* 1997;16:105-6.

70. Jeon Y-J, Fereidoon S, Kim S-K. Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. *Food Rev Int* 2000;16(2):159-76.

71. Pospieszny H, Mackowiak, Zolobowska, Struszczyk H. Effects of chitosan derivatives on the growth of phytopathogenic bacteria. In: *Progress on chemistry and application of chitin and its derivatives*. Polish Chitin Soc. Warsaw, editor Struszczyk, H, 1996, p. 101-6.

72. No H. Antibacterial activities of chitosans and chitosan oligomers with different molecular weights on spoilage bacteria isolated from Tofu. *J Food Sci* 2002;67(4):1511-4.

73. Pospieszny H, Mackowiak. Effect of the infection of plants by pathogenic bacteria Adv Chitin Sci 1997;2:759-62.
74. Li Y, Chen XG, Liu N, Liu CS, Liu CG, Meng XH, et al. Physicochemical characterization and antibacterial property of chitosan acetates. Carbohydr Polym 2007;67(2):227-32.
75. Rodríguez-Kabana R, Morgan-Jones G, Ownley-Gintis B. Effects of chitin amendments to soil on *Heterodera glycines*, microbial population and colonization of cyst by fungi. Nematropica 1984;14:9-25.
76. Brown FJ, Neville S, U. Sarathchandra, Watson RN, Cox NR. Effects of chitin amendment on plant growth, microbial populations and nematodes in soil. Newzealand Plant Prot.53:1-5.
77. Bélair G, Tremblay N. The influence of chitin-urea amendments applied to an organic soil on *Meloidogyne hapla* population and growth of green house tomatoes. Phytoprotection 1995;76(2):75-80.
78. Oka Y, Pivonia S. Effect of a nitrification inhibitor on nematocidal activity of organic and inorganic ammonia-releasing compounds against the root-knot nematode *Meloidogyne javanica*. Nematology 2003;5(4):505-13.
79. McCandliss R, Eastwood. B, Milch RA, inventors; IGI Biotechnology, Inc. (Columbia, MD) assignee. Nematocidally active chitin-protein complex US Patent 4,536,207. 1985 Aug 20.
80. Bhaskara MV. Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit. Postharvest Biol Technol 2000;20:39-51.
81. Ribeiro C, Vicente A, Teixeira JA, Miranda C. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol Technol 2007;44(1):63-70.
82. Molloy A, Cheah V, Koolard P. Induced resistance against *Sclerotinia sclerotium* in carrot treated with enzymatic hydrolyzed chitosan. Postharvest Biol Technol 2004;33:61-65.
83. El Ghaouth A, Wilson A, Benhamou N. Biochemical and cytochemical aspects of the interactions of chitosan with *Botrytis cinerea* in bell pepper. Postharvest Biol Technol 1997;12:183-94.
84. Chien P, Sheu F, Lin H. Quality assessment of low molecular weight chitosan coating on sliced red pitayas. J Food Eng 2007;79(2):736-40.
85. El Ghaouth A, Arul J, Asselin A. Potential use of chitosan in postharvest preservation of fruits and vegetables. In: Advances in Chitin and Chitosan, Brines CJ, Sandfors PA and Zikakis JP (Eds.). Elsevier Applied Science, London, New York, 1992, p. 440-52
86. Choquer M, Becker HF, Vidal-Cros A. Identification of two group A chitinase genes in *Botrytis cinerea* which are differentially induced by exogenous chitin. Mycol Res 2007;111(5):615-25.
87. Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS. Bioprospecting and antifungal potential of chitinolytic microorganisms. African J Biotechnol 2006;5(2):54-72.
88. Rafferty S, John M, Murphy G, Cassells AC. Lytic enzyme activity in peat is increased by substrate amendment with chitin: implications for the control of *Phytophthora fragariae* in *fragaria vesca*. Folia Geobot 2003;38:139-44.
89. Gomes RC, Semêdo LT, Soares RM, Alviano CS, Linhares LF, Coelho RR. Chitinolytic activity of actinomycetes from a cerrado soil and their potential in biocontrol. Lett Appl Microbiol 2000;30:146-50.
90. DeBoer, Gerards WS, Gunnewiek PJA, Modderman R. Response of the chitinolytic microbial community to chitin amendments of dune soils. Biol Fert Soils 1999;29(2):170-7.
91. Manucharova O, Yaroslavtsev AM, Senchenko DV, Stepanov AL, Zvyagintsev DG. Microbial transformation of chitin in soil under anaerobic conditions. Biol Bull 2006;33(2):191-4.
92. Manucharova NA, Belova EV, Vorob'ev AV, Polianskaia LM, Stepanov AL. Succession of chitinolytic microorganisms in Chernozem soil. Mikrobiologia 2005;74(5):693-8.
93. Leake JR, Read DJ. Chitin as a nitrogen source for mycorrhizal fungi. Mycol Res 1990;94:993-5.
94. Hidalgo L, Argüelles W, Peniche C. Efecto de la quitosana en tratamientos a la semilla de tomate. Rev Protección Veg 1996;11(1):37-9.
95. Freepons DE, inventor. Plant growth regulators derived from chitin. US Patent No. 4,964,894. 1990 Oct 23.
96. Rodríguez Y, Noval B, Ramírez M, Rodríguez P. Efecto de diferentes fuentes de quitina en el crecimiento de plántulas de tomate. Cultiv Tropic 1998;19(3):32-6.

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