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 1 ½, 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 form 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 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

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, especially 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 x 10^6 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 echinoderms [8].

Chitin is a white, partially crystalline, odorless and tasteless solid at its pure state. It is made of N-acetyl-2-amino-2-deoxy-D-glucose aminosaccharide units, linked together by β(1→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 bioproduct 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 cooper, zinc 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
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 antagonistic 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 antifungal 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 antifungal 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 or limiting disease progression. 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 solani 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 colloidal. 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 enhances the effectiveness of these compounds.

Chitin and its derivatives. Agricultural applications

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

<table>
<thead>
<tr>
<th>Use</th>
<th>Crop</th>
<th>Properties</th>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protection after harvest</td>
<td>Mango</td>
<td>Antimicrobial</td>
<td>Chitosan</td>
<td>[37-39]</td>
</tr>
<tr>
<td>Retardation of fruit ripening process</td>
<td>Papaya</td>
<td>Semipermeable film formation</td>
<td>Chitosan</td>
<td>[40]</td>
</tr>
<tr>
<td>Defensive enzymes stimulation</td>
<td>Rice</td>
<td>Inducer</td>
<td>Chitin</td>
<td>[41-43]</td>
</tr>
<tr>
<td>Mycorrizal symbiosis stimulator</td>
<td>Tomato</td>
<td>Inducer of recognition mechanisms</td>
<td>Chitin</td>
<td>[44]</td>
</tr>
<tr>
<td>Nematocidal control</td>
<td>Tomato</td>
<td>Increases soil chitinolytic microbiotes</td>
<td>Chitin</td>
<td>[45]</td>
</tr>
<tr>
<td>Biocontrol action enhancer</td>
<td>Peanut</td>
<td>Stimulator substrate for hydrolyases enzymes</td>
<td>Chitin</td>
<td>[46]</td>
</tr>
</tbody>
</table>

noses 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 *Phythophtora parasitica* is affected at low chitosan concentrations (i.e., 100 mg/L), while other require higher concentrations for significant inhibition (as *Fusarium oxysporum radicis lycopersici*, 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 fungi [52].

Results from investigations in rice demonstrated that seeds recovered with chitin derivatives increase hydrolytic enzymes production, such as chitinases and \( \beta 1,3 \) glycanases which degrade chitin and 1,3 glycanases, 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 oryzalexins, 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 are 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 *Phytiun aphidinumatum* [57]. In tomato, partially acetylated chitosans delivered either by seed or foliar routes induced hydrolytic enzyme production and reduced *Fusarium oxysporum lycopersici*-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 \( \beta 1,3 \) glycanase, chitinase and per-oxidases [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 infections. 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 molecular weight correlates with antiviral activity, since acetylation of chitosan. 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.
Nematicidal activity

Nematodes have been effectively controlled 0.008 aby 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 production, at concentrations toxic enough for nematodes, decreasing its population [77]. This last effect was corroborated by adding nitrogen fertilizers, which protect soil-added chitin from degradation and further reduce 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 prevails 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 Heterogena 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-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 eukaryote microbial populations and their activities, 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].

**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 microorganisms 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 microorganisms, to control pathogenic organisms and to strengthen plant nutrition, all these showing better results [38].

**Enhanced biopesticides**

Many antagonist organisms and natural biological controls exert their biological activity through chitina 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 fertile 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.
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, modulation 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 polymeric, 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 nematicidal 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.


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(s):615-25.


