Cultivos Tropicales, 2015, vol. 36, special edition, pp. 111-129

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

Review
NEW NATURAL PRODUCTS FOR AGRICULTURE: THE OLIGOSACCHARINS

Reseña bibliográfica
Nuevos productos naturales para la agricultura: las oligosacarinas

Alejandro B. Falcón Rodríguez✉**, Daimy Costales Menéndez, Dianevys González-Peña Fundora and María C. Nápoles García**

ABSTRACT. Oligosaccharins are natural polysaccharides and oligosaccharides occurring as part of cell walls of plants and microorganisms such as fungi; however, main sources of raw materials for its large scale preparation are by products from agriculture and wasted crustacean exoskeletons from fishing industry. They have potential agricultural applications since they promote germination and plant growth, enhance crop yields and benefit symbiosis in leguminous. A great number of studies demonstrate crop protection by oligosaccharins against biotic and abiotic stress. Some oligosaccharins, such as chitosans, perform direct antimicrobial activity, this fact reinforce their application as protective agent of agricultural commodities quality. There are several international commercial products based on these macromolecules that bearing also, as an additional valor, the innocuous and biodegradable features of these compounds. The Group of Bioactive Products (GPB) from INCA has developed several oligosaccharins based products that constitute national alternatives to agro-products as plant growth regulators, enhancers of crop yields, plant protecting agents against biotic and abiotic stress and new type of biofertilizers for biological nitrogen fixation in leguminous.

Key words: stress, fungus, microorganisms, oligogalacturonide, products, chitosan

RESUMEN. Las oligosacarinas son polisacáridos y oligosacáridos naturales que forman parte de las paredes celulares de las plantas y microorganismos como los hongos; sin embargo, las principales fuentes de materia prima para su preparación a gran escala lo constituyen subproductos agrícolas y el exoesqueleto de los crustáceos que se desechan de la industria pesquera. Poseen potenciales aplicaciones agrícolas, ya que promueven la germinación, el crecimiento de las plantas, el incremento de los rendimientos y el beneficio de la simbiosis de las leguminosas. Numerosos estudios demuestran la protección de los cultivos con oligosacarinas ante diferentes manifestaciones del estrés biótico y abiótico. Algunas como las quitosanas ejercen acción antimicrobiana directa, lo que eleva sus aplicaciones como agente protector de la calidad de las producciones agrícolas. Existen varios productos internacionales basados en estas macromoléculas que ostentan además, como valor agregado, la inocuidad y biodegradabilidad característica de estos compuestos. El Grupo de Productos Bioactivos (GPB) del Instituto Nacional de Ciencias Agrícolas (INCA) ha desarrollado varios productos a base de oligosacarinas que constituyen alternativas nacionales a productos agrícolas, como reguladores del crecimiento y los rendimientos, protectores de los cultivos contra el estrés biótico y abiótico y biofertilizantes de nuevo tipo para la fijación biológica del nitrógeno en las leguminosas.

Palabras clave: estrés, hongos, microorganismos, oligogalacturónido, productos, quitosano

INTRODUCTION

Today, there is a growing global need for food production, due to its shortage in certain geographical areas and to price rises and production costs, which are prohibitive for many third world countries. A great portion of agrochemicals used at present have high world market prices, which leads to high agricultural production costs. In addition, yet most of the chemicals employed to protect crops from diseases and some of them that improve productive efficiency are considered contaminating agents

Instituto Nacional de Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700.

✉ alfalcon@inca.edu.cu

of soils, crops and biodiversity, causing animal and human diseases.

Modern science in the latest 20 years has been designed to search for solutions and alternatives to those problems that may keep agricultural production efficiency. Therefore, the study of several branches of plant biology has deepened in plant mechanisms against different natural and human challenges, such as various biotic and abiotic stresses to which crops are subjected today. Results of the last two decades glimpse the development of a new generation of innocuous or less aggressive compounds to the environment and the man; their usefulness is based on managing natural plant responses against different stresses and maximizing inherent crop potentialities to raise yields.

This review deals with the characteristics and potentialities of a group of innocuous compounds (oligosaccharins) derived from nature with good prospects in organic and/or sustainable agriculture or even in a large-scale or intensive agriculture.

OLIGOSACCHARINS

They were discovered as a result of studies made on two very important plant biology issues during the 70s and 80s. Firstly, multiple groups of the first world studied plant-microorganism interaction, particularly plant responses to pathogens and predators, as well as signals related to these responses; secondly, a few researchers guided by Dr. Peter Albersheim in Georgia, USA, studied plant cell wall structure and components under suspicion of its complexity, for its functions in the plant were probably not only to support, but also its content shape and protection.

Both lines converged on very important results that caused a revolution of concepts and views in both issues. Thus, it is now known that plant cell wall is not only a reservoir or cell support, but a deposit of hormones acting on a wide range of plant functions, maybe directly or indirectly on the so-called traditional hormones and, especially, activating plant defense and resistance responses against pathogens and predators.

CONCEPTS: ELICITORS AND OLIGOSACCHARINS

Plants have the ability to defend themselves from most microorganisms, potentially pathogens, living in their environment. Crops generally have structural barriers and chemical compounds preventing infection progress; apart from these preset defensive mechanisms, plants can induce the expression of many defense genes, both locally and systemically in all tissues, with a coordinated action that holds up any disease establishment (1, 2).

Plant defense response is the result of recognizing various compounds released by pathogens and the plant itself during pathogenesis process, when enzymes excreted by both contenders degrade cell walls of the other organism (3, 4, 5). Released structures recognized by the plant are called elicitors.

Elicitors are substances that can induce defensive responses when added in plant tissues or cells. They are compounds of diverse structure and origin: oligosaccharides, glycoproteins, peptides, lipids, among others. It has been shown that elicitors of oligosaccharide type have significant roles in plant-pathogen interactions (3).

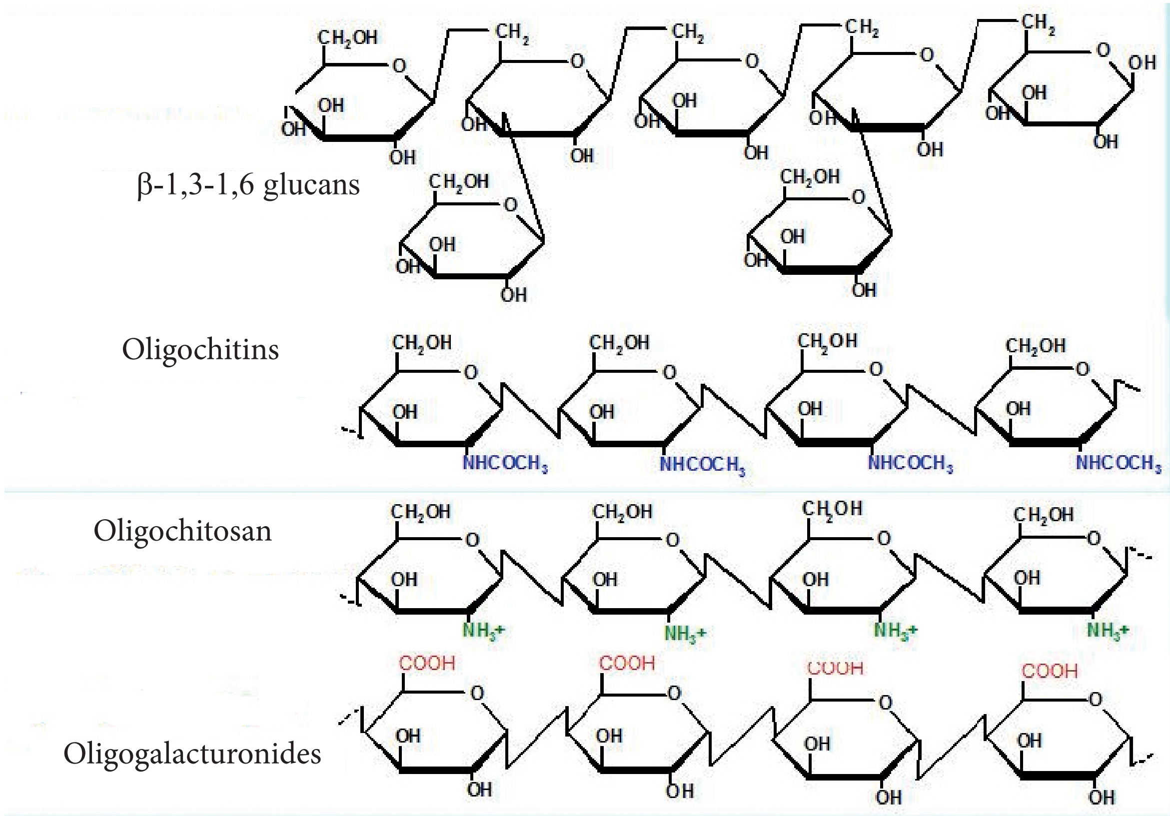
Polysaccharide and glycoproteic components from cell walls constitute a source of oligosaccharides, which besides being elicitors of plant defense responses, some of them have effects on plant growth and development at low concentrations. Therefore, the term **oligosaccharins** refers to oligosaccharides of different origin with biological effects on plants. They consist of a chain of glycoside residues linked by glycosidic bonds (6, 7).

Types, classification, localization and structure

Oligosaccharins are of endogenous or exogenous type, depending on the way they are obtained or released from cell walls of the plant or pathogen, respectively. According to its origin, there are different types (Figure).

Endogenous oligosaccharins

Oligogalacturonides (pectic oligosaccharides) and xyloglucans are among the most widely known and studied endogenous oligosaccharins (Figure).



Structure of the main oligosaccharins released during pathogenesis process and related to plant resistance induction against pathogens

Oligogalacturonides consist of a linear chain of galacturonic acid molecules linked by α-1-4 bonds. The number of D-galacturonate residues contained in an oligosaccharide defines its degree of polymerization (5). They are located in the pectic portion of plant cell wall and are naturally released from pectin through enzymatic hydrolysis of plant action or as a result of pathogen attack (4, 5).

Meanwhile, *xyloglucan* polymers are major hemicellulosic polysaccharides making up the primary cell wall structure of non-poaceas dicot and monocot plants. They also form part of reserve polysaccharides in dicot seeds. They consist of a skeleton of glucose residues linked by β-1-4 bonds; some of these residues may be replaced by α-xylose, β-galactose and α-Fucose (8). These polymers fragmented by chemical or enzymatic hydrolysis release xyloglucan oligosaccharides with plant biological activity (9, 10). Specifically, the phenomenon of growth and extension at cellular level is closely related to xyloglucan polymer metabolism whereas its enzymatic degradation causes plant cell wall weakening, besides releasing the above mentioned fragments, (11, 12).

Exogenous oligosaccharins

Oligoglucans, Oligochitins, Poli and Oligochitosans and Lipo-chitin-oligosaccharides are known among exogenous oligosaccharins (Figure).

Chitin derivatives and oligoglucans are released from the cell wall of various pathogens containing them, through enzymatic degradation of glucanase and chitinase enzymes excreted by the plant in response to pathogen attack during pathogenesis process (4, 7). In turn, Nod factors (Lipo-chitin-oligo) are de novo synthesized and excreted by Rizobiaceae bacteria, in response to chemical signals released by the plant and detected by the microorganism (13).

Other sources of production

Despite the origin above mentioned, oligosaccharins can be extracted from other richer sources contained in polysaccharides; thus, commercial citrus pectin is the main source to obtain oligogalacturonides while tamarind seeds are rich in xyloglucans. Crustacean exoskeleton is very rich in chitin and the permanent natural presence of this chemical structure in biosphere is of 10 gigatons (10^{13} kg) (14). Because of the versatile applications of its derivatives, mainly chitosan and glucosamine, chitin is essentially produced on an industrial scale from crab, shrimp, lobster and prawn, at about 10,000 tons per year (14, 15).

Several worldwide research groups and agricultural enterprises have started to develop alternative agrochemicals based on oligosaccharins; the choice of an appropriate source for obtaining polymers and oligosaccharides is one of the key factors in reducing production costs and sale prices for different agricultural systems. Thus, 90% chitin and chitosan-based products come from exoskeleton of million tons of crustaceans caught on a world scale that constitute industry wastes (15).

Another example is a commercially sold β-glucan product. A major French company "GOEMAR" together with the National Center for Scientific Researches of France created a laminarin-based product, Iodus 40Ò (Vacciplant), extracted from seaweed, which activates intrinsic plant protection against potential pathogens when applied preventively to various commercially important crops.

In Cuba, the Group of Bioactive Products (GBP), pertaining to the National Institute of Agricultural Sciences (INCA), made a methodology for obtaining a mixture of biologically active plant oligogalacturonides, derived from commercial citrus pectin, named "Pectimorf" (16) and, at present, they are developing another methodology to prepare a chitosan-based product, derived from chitin of Cuban lobster exoskeleton wastes of fishing industry. Both products have shown different biological effects on economically interesting crops[A] (16).

BIOLOGICAL EFFECTS OF OLIGOSACCHARINS

Oligosaccharines were first recognized as polysaccharides and oligosaccharides that induced defensive responses and resistance to plants. However, subsequent studies performed in the 90s involved them in several responses related to plant growth and development (6, 17). The discovery of the main carbon skeleton structure from nod factors excreted by Rizobiaceae bacteria and its effect on legume root morphogenesis helped establish oligosaccharins as a new hormone hierarchy in plants, whose action precedes the synthesis and accumulation of already known traditional hormones (6, 18, 19).

Regulation of plant growth and development

Exogenous application of oligosaccharins influences plant tissue growth and development; such evidence has been essentially obtained with oligosaccharides derived from plant cell wall polymers, also with chitin and chitosan derivatives[1] (20, 21). Table I presents some examples of endogenous and exogenous oligosaccharin effects in commercially important crops[B] (22, 23, 24, 25, 26, 27).

Among endogenous oligosaccharins or derived from plant cell walls, oligogalacturonides or oligopectates have been most widely studied for their effect on plant growth and development. In many cases studied, its effect on the plant seems to be opposite to auxin action.

The negative effect of oligogalacturonides of different degrees of polymerization (DP) was proved in pea stem elongation induced by indole acetic acid (IAA), inhibition of root formation in thin cell layers growing inside rooting culture medium, reduction of a protein accumulation in culture medium induced by certain IAA levels in the presence of oligopectates in the medium and inhibition of cell division induced by auxins in phloem parenchymatous cells (5, 28). However, further results of a mixture of oligopectates commercially known as Pectimorf (Pm) included in an *in vitro* medium culture of different species, with certain plant hormonal balance, indicate an auxin effect based on rooting stimulation, bud increase and plant growth (21, 22, 29).

[A] Falcón, A. B.; Cabrera, J. C.; Reinaldo, I. M. y Nuñez, M. N. *Desarrollo de activadores de las plantas de amplio espectro de acción*. Informe Final del PNCT, no. 00100191, Inst. CITMA, Cuba, 2005.

[B] Falcón, A. B. Evaluación de Oligosacarinas nacionales de quitosana en la estimulación del crecimiento, la nodulación y la protección de cultivos de interés económico. Informe Final del PNCT, no. 00300277, Inst. CITMA, 2009, p. 77.

Tabla I. Efecto de oligosacarinas sobre el crecimiento, el desarrollo, los rendimientos y la calidad poscosecha de diferentes cultivos

Cultivation	Effext iobserved in different types of applications	References
α 1-4 Oligogalacturonides (oligopectates)		
Sugar cane, banana	Hormone replacement by Pectimorf (Pm) *in vitro* culture increases the number of shoots, rooting and benefits subsequent acclimatization process vitroplant.	22, 23, 24
Grape	Foliar spraying of a mixture of oligogalacturonides in clusters prior to ripening grapes cause increased coloration and anthocyanin content in the fruit.	25
Areca palm	The double foliar spray the mixture Pm (2 mg L^{-1}) increases growth and reduces time areca plants aviveramiento.	26
Lettuce radish	The foliar spray the mixture increases Pm air mass in lettuce and root length and shoot and root mass radish.	27, 28
Tomato	Imbibition of seeds with Pm and its combination with mycorrhiza increased the rooting of seedlings. The foliar spray increases growth and increases crop yields.	A, 28
Oligosaccharides xyloglucans		
Arabidopsis thaliana	0.1 mg L^{-1} benefits primary root elongation coupled with a slowdown in the formation of lateral roots.	34, 35
Tobacco (cell line BY-2)	Shortening the cell cycle through the reduction step G1 in mitosis.	35, 36
Arabidopsis thaliana	Stimulate growth and reversed large and small cellular phenotypes that normally exhibit $WEE1^{oe}$ and $Spcdc^{25}$ genotypes, respectively.	35,36
Chitosan. Application imbibition coating or seed		
Sunflower	MM different chitosan. immersed for 18 h in the lower MM (28 kDa) increased total seed caused shoot mass, increased germination and isoflavonoids level.	42
Pearl Millet	Increased growth Millo.	43
Corn, Wheat	Increase germination quality and vigor of the positions.	44, 45
Peanut	Increased germination, lipase activity and levels of AG y AIA.	46
Rice	Imbibing seeds + application to the substrate significantly increases performance.	47
Cotton	Coating 0,2 % for 30 minutes caused the greatest increases in plant height and fruit yield.	48
Chitosan. Foliar spray application		
Soja, Corn	Applications of chitin and chitosan pentamer causes variations in photosynthesis, stomatal conductance, transpiration and [CO_2] intercellular.	49
Strawberries	Four foliar applications of chitosan caused increased height, number and fresh weight and dry leaves and yield (number and mass).	50
Tomato, Lettuce	Application of chitosan 100 kDa 0,1 % growth and increases yields. 50 % increase in the leaf area of lettuce.	48
Potato	Sprinkling of chitosan increases the performance and quality of mini tubers, as well as growth and yields in field experiments.	52, C
Tobacco, Tomato	Chitosan polymer dispersion increases growth and yields in experiments and production scale extensions.	B, C

In addition, *ex vitro* results have been obtained with the same mixture related to root formation at similar levels to those induced with IAA[A] (30).

A potential application of oligopectates (and maybe of other oligosaccharins) is to improve color in some fruits. Recently, a research group studied vine color response when applying a mixture of oligogalacturonides with lower degree of polymerization than 20 on a production scale. Results showed significant color increases over the control and commercial product (Ethephon) used for this purpose. Such increase was directly related to higher anthocyanin contents (responsible for vine color) and also with gene expression of phenylalanine ammonia lyase (PAL) within the early days of application, an enzyme that opens multiple enzymatic pathways, some of them leading to anthocyanin formation (24). This correlation supports the possibility of other PAL inducers to increase grape color.

Specific xyloglucan fragments show anti-auxin or auxin activity depending on the type of monosaccharide residues linked to oligomer chain of xyloglucan and the concentration used (31, 32). Its effects at nanomolar concentrations make them primary signals to regulate hormones.

More recent studies with xyloglucans coming from tamarind seeds showed effects in promoting primary root growth interacting with reduced lateral root formation (33, 34), as well as shortening cell cycle, particularly at G1 mitosis phase and reversal occurrence of larger or smaller sizes of cell phenotypes obtained with particular genotypes of *Arabidopsis thaliana* (34, 35).

Meanwhile, both chitosan polymer and its smaller derivatives are considered plant growth and development regulators when stimulating root and vegetative growth of several species (20, 36), shortening flowering period and improving flowering and fruiting (37, 38). Higher yields and crop quality have been observed in several crops with these derivatives, which have allowed them to be patented for this purpose (36, 39). In general, depending on plant organ in question, the aforementioned beneficial results have been achieved when treating seeds and plant roots or by spraying leaves at the right times for each crop[B] (36, 39, 40).

In turn, exogenous plant applications, mainly with oligogalacturonides and chitosans (Table I) on a field and greenhouse scale, have shown that such oligosaccharins influence growth and yields of economically important species from Solanaceas, Cucurbitaceous, Poaceas and Fabaceas, among other families[A, B,C] (25, 26, 27, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 50). Some authors suggest the positive impact on plant growth is related to an antitranspirant effect induced by stomatal closure (51, 52).

According to some studies, reducing irrigation with chitosan to pepper plants allowed a better adaptation and water consumption by the plant, since foliar application of the polymer reduced water use by plants between 26 and 46%, whereas biomass production and yield remained similar to control plants that were not subjected to water deficit (53). This occurred by decreasing water loss through stomata, due to stomatal closure induced by chitosan. This stomatal closure was subsequently studied, showing a higher abscisic acid in chitosan-sprayed leaf cells, which reduced stomatal conductance (52). This supports the use of chitosan as an antitranspirant to preserve water applied to agriculture.

Additionally, synthesized oligosaccharins and excreted by nitrogen-fixing rhizobacteria from Rhizobiaceae family cause seed germination of various plants and are involved in primary root events leading to establish symbiosis between legumes and mentioned bacteria (18, 54, 55). These oligosaccharin structures consist of chitin oligosaccharides of 4 or 5 N-acetyl glucosamine residues linked to other complex groups varying with *Rhizobium* species. These oligochitins are responsible for inducing root cortical cell divisions that initiate and lead to subsequent nodule formation (56).

INCA's GBP studied chitosan applications to *in vitro* soybean experiments combined with *Bradyrhizobium elkanii* micro-symbiont. By including chitosans of different molecular mass to plant growing medium, increased the number of nodules and its dry mass formed in the root depending

[C] Falcón, A. B. Compuestos de quitosana como activadores del metabolismo, el crecimiento y la resistencia contra el estrés biótico en cultivos de interés económico. Informe Final del PNCT, no. 00300330, Inst. CITMA, 2012, 70 p.

on the concentrations used. An effect was also observed on seedling growth and root volume, depending on the concentration. Concentrations above 500 mg L^{-1} did not benefit root growth or plant aerial system[D] (57, 58).

INDUCING PLANT RESISTANCE AGAINST PATHOGENS

b-Glucan oligosaccharides

Oligosaccharides derived from β-glucan polymers making up cell walls of *Phytophthora* pathogens have been established as inducers of plant defensive responses. Thus, a branched hepta-β-glucoside, obtained from wall glucan of *Phytophthora sojae* by acid hydrolysis, proved to be a very active elicitor of phytoalexin-gliceoline synthesis in soybean cotyledon cells (59), so demonstrating that partial glucan hydrolysis of *P. sojae* released fragments with elicitor activity in different plants of Fabaceae (60) family, indicating a similar system of perception in this plant family.

In tobacco cells, hepta-β-glucoside did not act as elicitor; however, a linear chain of β 1-3 glucan (laminarin), extracted from *Laminaria digitata* alga, proved to be an active elicitor of defensive responses (61) and even reduced vine leaf fungal infection (62). Regarding the above mentioned results (Table II), the French company GOEMAR, together with the National Center for Scientific Researches, developed a laminarin-based product, Iodus 40® (Vacciplant), extracted from seaweed, which activates intrinsic plant protection against potential pathogens when applied preventively.

Pectic oligosaccharides or oligogalacturonides

Oligogalacturonides derived from pectic polysaccharides of plant cell walls have been described as inducers of a great variety of defensive responses in cells, organs and whole plants of many species, among which are phytoalexin induction, proteinase inhibitors, PR proteins and lignification process (5, 7).

Oligogalacturonides can be generated from pectic substances of the primary plant cell wall by partial acid hydrolysis or by the action of pectinase enzymes or pathogen pectatoliases. Moreover, it is known that, regardless of the generation method, they depend on the degree of polymerization (DP) for inducing defensive responses, the most active DP being between 10 and 12 (5, 7).

In the latest decade, protection of two commercially important species (Table II) against its major pathogens was reported by inducing resistance with oligogalacturónidos[A] (63). It is essential to note that, unlike chitosans, oligogalacturonides and β-glucans do not have direct antimicrobial action on microorganisms, so that their crop protection results from activating induced plant resistance.

Polymers and chitosan oligosaccharides

Chitin, a polymer of N-acetyl-glucosamine linked by β 1-4 bonds, is a common cell wall component of several fungal families (64). Its fragments (N-acetyl-chito-oligosaccharides) have been involved in inducing a variety of plant defense responses, such as phytoalexin induction, lignification, PR proteins, defensive gene expression, etc., mainly in monocot species and especially in rice cell suspensions (65).

Besides, the activation of secondary responses related to defensive signal transduction by chitin fragments has also been studied, highlighting changes in ion flux and protein phosphorylation, depolarization of plasma membrane and K^+ and Cl^- ion efflux, cytoplasmic acidification, generation of reactive oxygen species and biosynthesis of jasmonic acid (7, 65, 66).

Chitosan is a polymer of β 1-4 glucosamine, a natural cell wall component of Zygomycete fungi (64). Both the polymer and its oligomers are potent inducers of defensive responses and plant resistance against pathogens. However, the concentrations required for activating defensive responses are higher than those needed to induce such activities with chitin oligomers (7, 65).

[D] Costales, D. Quitosacáridos en la nodulación y el crecimiento de soya (*Glycine max* (L) Merril) inoculada con *Bradyrhizobium elkanii*. Tesis de Maestría, Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba, 2010, 56 p.

Tabla II. Resultados de protección de diferentes especies de plantas contra patógenos por oligosacarinas

Culture	Protective effect observed	Reference
β 1-3 glucans		
Grapes	Fragment of laminaria (β glucan 1-3) cause induction of defensive response and resistance against *Botrytis cinerea* and *Plasmopara viticola.*	63, 64
α 1-4 oligogalacturonide		
Grapes	Induction signals and defensive responses against *Botrytis cinerea* Vid.	65
Tobacco	Induction of defense responses and resistance against *Phytophthora nicotianae* with Pm.	A
Chitosan-Protection postharvest-Frutales		
Papaya, Mango	Protección de la fruta vs. la Antracnosis (*Colletotrichum gloeosporioides*).	87, 88
Peaches, Pears and Kiwi	Reduction fungal rots in storage of these fruits when covered with chitosan.	89
Citrus	Protection vs. *Penicillium* and *Botrytis* fruit by coating chitosan.	90
Strawberry	Coating fruit with chitosan and its combination with Ca^{2+} reduces incidence of pathogenic fungi, reduces weight loss and increases firmness and shelf life.	91
Chitosan-protection food crops postharvest-interest		
Rice	Or protection. Blight (*Pyricularia grisea*) by Poli and chitosan oligo.	92, 93
Vid	Protection and fruits or leaves gray mold (*Botrytis cinerea*) and poly oligoquitosanas.	94, 95
Cucumber	Protection vs. gray mold (*Botrytis cinerea*) with chitosan polymer.	96
Carrot	Root protection vs. *Slerotinia sclerotiorum* with hydrolyzed chitosan.	97
Tomato	Plant protection vs. *Fusarium oxysporum* and fruit vs. soft rot caused by *Rhizopus* sp.	98, 99
Tomato	Plant protection against infection by *Xanthomonas gardneri.*	100
Chitosan-crops of economic interest		
Tobacco	Protection vs. Black shank (*Phytophthora nicotianae*) of systemic resistance induction by application of chitosans of different characteristics.	72, 75
Tobacco	Protection vs. mosaic virus (TMV) and necrosis virus (TNV) with oligoquitosanas and its relationship with the ABA.	101, 102

As in the case of oligogalacturonides, chitin fragments (and also those of chitosan) are dependent on the degree of molecular polymerization (DP) to activate the aforementioned defensive responses, its sizes being above DP4, and mainly heptamer and octamer, which elicit responses at lower concentrations (7, 66).

Chitosan is the most studied oligosaccharin with more applications in pre- and post-harvest field of agriculture. It has three essential characteristics of biological activity that makes it desirable in this field: It improves growth and yields of many crops; it induces plant defense and resistance against pathogens and, unlike other oligosaccharins studied, it generally inhibits microorganism growth and development (16, 67, 68, 69, 70). Moreover, its biological activity is related to free positive charges present at the amino group under acid conditions, interacting with opposite charges of cell wall components and membranes of microorganisms and plants.

Chitosan antimicrobial activity

Chitosan antimicrobial activity has been established both *in vitro* and *in situ* experiments (14, 67). Literature reports that growth inhibition of many pathogens, including fungi, bacteria and oomycetes, is highly correlated with increased chitosan concentration in culture medium, indicating that a higher concentration causes a greater inhibition, so there are differences between fungistatic and fungicidal action, according to tested concentrations (Table III).

Tabla III. Actividad antimicrobiana de quitosanas y oligoquitosanas en diferentes microorganismos

Microbial species	Antimicrobial effect observed	Reference
Bacterium		
Escherichia coli	Reduction in cell viability.	124
Bacillus subtilis	Reduction in cell viability.	124
Staphylococcus aureus, S. simulans	It caused involvement of cell viability and permeabilization and cell membrane depolarization.	125
Bradyrhizobium elkanii	Inhibition of cell viability at concentrations above 0,5 g L^{-1}.	D
Fungi		
Fusarium oxysporum, F.solani	Inhibition of mycelial growth and spore germination.	77, 126
Botrytis cinerea	Mycelium growth inhibition, germination spore, germ tube elongation and causes damage to the membrane spores.	79
Alternaria alternata, solani	Inhibition of mycelial growth and formation spore.	80
Aspergillus niger	Inhibition of mycelial growth and spore germination.	81
Rhizopus stolonifer	Chitosans of different MM affect vegetative growth, sporulation and spore germination	88, 127
Penicillium digitatum y expansum	Affectation mycelium, reduced viability of the spores and damaged cell membranes.	69, 79
Pochonia chlamydosporia (nematófago), *Beauveria bassiana* (entomopatógeno), *Trichoderma harzianum* (micoparásito)	Inhibition of vegetative growth and spore germination with different degrees of involvement for each gender. Vegetative sensitivity: Trichoderma>Fusarium>Pochonia>Beauveria Sensibilidad en germinación de esporas: Trichoderma= Fusarium>Pochonia>Beauveria	77
Oomycetes		
Phytophthora capsici, P. nicotianae y *P. palmivora*	Inhibition of vegetative growth and different stages of the life cycle as sexual reproduction and asexual.	17, 72, 73, 74, 75, E
Pythium aphanidermatum	Reduced radial vegetative growth and infection in cucumber.	76, 83

For more than two decades, these compounds have been evaluated over all fungal stages of life cycle, its reproductive structures being mostly affected (14, 67).

The study of chitosan effect on the group of oomycetes is more recent. Some authors have shown that chitosan polymers affect the vegetative development of *Phytophthora* isolates. Higher concentration depending on the species causes a significant colony growth reduction, which has been observed in species such as *P. nicotianae*, *P. capsici* and *P. palmivora*; all of them are important pathogens of several plant species[5] (71, 72, 73, 74). Some of these species are more sensitive than others; for example, *P. nicotianae* and *P. capsici* reduced over 50 % their vegetative growth with about 0,5 g L^{-1} (71, 73, 74), while *P. palmivora* needed more than 2 g L^{-1}. Between oomycetes and true fungi, results generally show an apparent higher sensitivity in the first ones, as observed by comparing experiments between *Phytophthora* and *Pythium* against species of different fungal groups; some nematophagous and entomopathogenic fungi are even much less sensitive (71, 74, 75).

Other stages of microorganisms, as reproductive and asexual dispersion structures, may be more sensitive than those of the vegetative growth. Inhibition of spore germination by chitosan has been observed in many fungi, such as *Aspergillus niger*, *Alternaria alternata*, *Rhizopus stolonifer*, *Mucor* sp. and *Pochonia chlamydosporia* (74, 75, 76, 77, 78, 79, 80).

[E] González, P. D. Efecto de un polímero de quitosana en el desarrollo de *Phytophthora nicotianae* y *Phytophthora palmivora*. Tesis de Maestría, Facultad de Biología, Universidad de la Habana, La Habana, Cuba, 2011, 50 p.

Within oomycetes, *Phytophthora* has been the most studied genus. Chitosan polymers and oligomers inhibit zoospore and zoosporangia formation and germination in *P. capsici*, *P. nicotianae* and *P. palmivora*[E] (16, 71, 72, 73).

Physic-chemical properties of these compounds also inhibit microorganism growth. For example, when polymer molecular mass and degree of acetylation decrease, mycelial growth inhibition and spore germination of *Phytophthora nicotianae* increase (16, 73). Similarly, in *Rhizopus stolonifer*, variations from 1.0 to 2,0 g L^{-1} did not modify spore formation or germination; however, inhibition of these processes was observed when using chitosans of different molecular mass (76).

It is suggested that chitosan antimicrobial activity is mainly due to polycationic character of the molecule in solutions at pH below 6.0, since positively-charged amino groups can interact with phospholipids from cell membranes of microorganisms and modify its permeability. This may cause osmotic imbalances that lead to structural disruptions and eventually to cell lysis (67, 81). For the case of oligochitosans, the internalization of these molecules in microbial cell was proved and its possible interaction with DNA is speculated (72). The amount of damage found can vary, mainly depending on the physic-chemical properties of the polymer and concentrations employed (14, 67).

Despite many chitosan benefits shown in dozens of works and the increased number of patents for its applications in the latest 15 years, this polymer is not yet so exploited in the global agricultural context. In fact, most agricultural chitosan-based products (Elexa®, Chitogel®, Aminogro®, Chito-Plant®, Chito-Care®, etc.) started to appear about a decade ago and, so far, they do not have a high demand or production (41, 82). However, a recent boost of chitosan evaluations has been noticed under controlled, non-controlled and glasshouse conditions[A] (16, 41, 82, 83) and even its extension and assessment as a result of governmental decisions. At present, this polymer is known among biopesticides as a "crustacean-derived activator of plant defense" (84).

Plant protection by chitosans against biotic stress

Crop protection through chitosan applications against its main diseases have been studied for over 20 years in different plant-pathogen interactions by many research groups worldwide. Several examples of protection reported in distinct species and growing times are referred in Table II (70, 73, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100). Crop protection against pathogen attack may be due to the antimicrobial activity of these polymers and oligomers on microorganisms or may be it is the result of a higher basal resistance caused by activating resistance induced by these compounds in the plant. In many cases, both effects can occur simultaneously (16, 67, 69, 70).

Plant protection by oligosaccharins against abiotic stress

More recent studies have demonstrated potentialities of oligosaccharins in certain species protected against different forms of abiotic stress. Most studies refer to evaluations and results of the last decade with exogenous oligosaccharins; however, results with endogenous oligosaccharins have been published as patents.

In this sense, a process for adapting plants to different abiotic stresses was recently patented, including foliar spraying of a xyloglucan derivative under particular conditions of application. According to authors, this process benefits plant growth under various stresses, including low temperatures, drought, humidity or salinity, by stimulating enzymes and compounds which decrease the levels of reactive oxygen species released during stress and also widen some signals that lead to the formation and/or activation of traditional hormones acting in plant cell (101).

A growing number of protection results against abiotic stress have been reported in plants with different types of chitosan treatment. The studies range from controlled trials up to large-scale experiments in different crops. Meanwhile, applications can be by seed coating or soaking for a short time with high concentrations or the reverse, lower concentrations and longer contact times, depending on the type of seed. Also, good results were obtained with treatments by foliar spraying, applications to growth substrate or by introducing roots or cuttings in chitosan solutions. Various abiotic stresses have been tested with more or less success, depending on experimental conditions. Some examples of species protection against different forms of abiotic stress are summarized in Table IV (102, 103, 104, 105, 106).

ECOLOGICAL BENEFITS OF OLIGOSACCHARINS

The origin and oligosaccharide chemical structure of oligosaccharins talks about its possible non-toxic characteristics. Oligogalacturonides making up Pectimorf are extracted by enzymatic hydrolysis of citrus pectin, a widely used raw material in food industry, whose final product is biodegradable by soil microorganisms; once it is applied, no traces of the product are left on the plant or substrate[F].

Something similar happens with chitin and chitosan-based products; although it can be toxic to many soil phyto-pathogens, it is an excellent source of carbon for others. Moreover, chitin and chitosan products are widely used in human and veterinary medicine, cosmetics and industry, because it is safety for humans (15, 107).

Another important ecological contribution of both products is the fact that raw materials (industrial pectin and chitin), from which they come, constitute the added value of potentially polluting byproducts of food and fishing industries, respectively, as they are wastes of juice production and exoskeleton of crustaceans caught for human consumption. Therefore, the preparation of both products provides an economic and ecological output of both industrial byproducts.

RESULTS AND PROSPECTS FOR CUBAN OLIGOSACCHARIN-BASED PRODUCTS

INCA's GBP has a 20-year-old experience studying oligosaccharins, its preparation and effects on plants. The group develops products based on oligogalacturonides (Pectimorf®) and chitosans (QuitoMax, requested to CIPO), as principal active agents, using their own methodologies (16). Both products have different biological effects on plants and allow to be applied as alternative products for different agrochemicals.

Pectimorf (Pm) promotes plant root development at concentrations between 5 and 20 mg L^{-1}, which has been demonstrated in experiments with seeds and cuttings treated by foliar spraying and by combining the forms of applications mentioned in vegetables, fruit trees and ornamental plants (25, 26, 27). At present, this product is introduced and spread as rooter in agriculture throughout the country, and new presentations are being tested to enable its applications.

Increased leaf development and plant growth have also been observed in Solanaceae and legumes, as well as in soybean and bean yields (26, 27, 108). Likewise, a positive effect has also been shown on activating growth of slow-growing ornamental plants, such as Areca, Anturium and orchids, by foliar spraying of Pm at different concentrations and application times (25, 109, 110).

Pectimorf has been extensively studied *in vitro* culture, demonstrating its ability to substitute traditional hormones (auxin and cytokinins) at different stages and diverse crops: sugarcane, coffee, citrus, potato, tomato, tobacco, banana, rice, garlic, among others[F] (21, 22, 23, 29). In this case, there are some crop benefits as rooting promotion, increased buds and good results at *ex vitro* adaptative stage. Other results recorded with Pm are a delayed opening of freshly cut flowers, specifically roses, and increased buds in violets treated with product spraying[F].

Pectimorf has also been related to plant protection against different types of stress. For example, induction of defensive responses and protection against oomycetes (*Phytophthora*) has been evaluated in tomato and tobacco by seed treatment and foliar spraying[A].

[F] Cabrera, J. C. Obtención y purificación de oligogalacturónidos bioactivos a partir de la pectina cítrica. Informe Final del PNCT, no. 002, Inst. CITMA, La Habana, Cuba, 2000, p. 150.

Tabla IV. Resultados de protección contra el estrés abiótico inducido por quitosanas

Culture	Antistress effect observed in different types of applications	Reference
Rice	Chitosan solution in hydroponics reduced the presence of vanadium in stems and root buds allowing rooting and growth in treated seedlings stressed above control.	104
Lettuce, Onion, Tomato	Seed treatments and foliar spray combination with chitosan induces a complex salt tolerance during germination and growth.	105
Rice	The foliar spray prior Chitosan drought stress caused higher yields under such stress and a good recovery plants.	106
Grape (Vid)	Imbibition stakes in Chitosan induced tolerance against drought stress and low temperatures expressed as greater rooting, sprouting and increased leaf chlorophyll structures formed in multiplication.	107
Corn	Seed treatments induce tolerance to the growing plant under stress acid.	108

Regarding abiotic stress, the potential of this oligogalacturonide mixture was assessed in tomato plants protected against heavy metal stress and it was found that pre-soaked tomato seeds with Pm enabled to reduce its effect on seedlings growing in a copper-poisoned substrate, reaching similar growth values to the control grown in a non-metal substrate (111).

INCA's GBP has developed methodologies to prepare chitosan compounds with different physic-chemical characteristics. Thus, biological potentialities of partially hydrolyzed chitosan were studied in polymer and chitosan oligosaccharides, differing in their biological action, depending on the molecular mass and degree of acetylation of amino group. These compounds have been investigated as resistance inducers against pathogens in crops as tobacco, tomato, soybean and rice, as well as inhibitors of principal crop pathogens[D, E] (16, 73, 74, 112, 113, 114). On the other hand, their potentialities to increase crop growth and yields were also demonstrated in tobacco, tomato, corn and cucumber[B] (16, 115, 116).

Considering INCA's GBP's results, a chitosan formula known as QuitoMax (requested to CIPO) was developed, which is not yet registered as a liquid byproduct, but it is still being validated in the field through extensions and controls in different provinces of Cuba. In this sense, during 2013-2014 seasons, hundreds of hectares of potatoes and beans were extended in Mayabeque province whereas tens hectares of tobacco and tomato were extended in Granma province.

Studies performed when validating QuitoMax support the preparation of chitin and chitosan polymers from exoskeletons of Cuban lobster, which constitutes a polluting waste from fishing industry, in order to be applied on a greater scale in agriculture. Among the advantages for its use are: they are biodegradable and non-toxic compounds once they are released into the environment and can be obtained through non-polluting methods from domestic raw materials that constitute wastes (16). Also, its antimicrobial action against pathogens and compatibility, even its synergistic action with several biological controls should be added; its activation of induced resistance against further pathogen attacks, when previously applied to crops and its effect for promoting growth, vegetative development and yield[B] (16, 70, 73).

The latest results of GBP show increased growth and yields ranging from 10 to 60% above the controls, depending on the product (Pectimorf or QuitoMax) evaluated, different forms of applications tested in the crop and the location studied[A, B, C]. These promising results, some within extension phase, have been proved in crops as tobacco, tomato, potato, corn, rice, cucumber, soybean and beans. In the latter two crops, a synergistic effect of both products was separately shown with biological nitrogen-fixing microorganisms used as biofertilizers in such crops[C] (108).

The group has also designed and developed a new concept of biofertilizers for biological N fixation, based on the production of biological preparations with rhizobia and enriched by nodulation factors, synthesized oligosaccharins and excreted by these symbiont microorganisms of legumes (117, 118). By exploiting a greater presence of these macromolecules in such biological preparations, an added value was reached, that goes beyond a single bacterial biomass increase to propose a larger number of structures inducing the early events in nodule formation, where atmospheric nitrogen will be further fixed.

Comparing traditional biofertilizers for soybean to that enriched by nodulation factors (commercially known as Azofert) proved the advantages of the second one in root nodule formation and increased crop growth and yield (119). Other results show that the presence of these oligosaccharins in biological preparations causes greater soybean protection against biotic and abiotic stresses (120, 121).

The latest researches and validations of GBP conducted on a field scale glimpses, in promising bean and soybean results, a synergistic effect between exogenous and endogenous oligosaccharins with microbial inoculants, which allow considering the use of oligogalacturonides and chitosans for the synergistic or additive potentiation of such biofertilizer in the symbiotic process and growth of legumes.

CONCLUSIONS

- ♦ Oligosaccharins are natural not toxic and biodegradable compounds, with various biological effects on plants and microorganisms that make it attractive for its use in sustainable and intensive agriculture, replacing agrochemicals to protect plants against diseases and substituting growth regulators.
- ♦ Although they are structures that form part of cell walls from plants and microorganisms, there are other commercial or industrial waste sources, allowing a cheaper production, which can be performed by chemical, physical and enzymatic methods which are not expensive.
- ♦ Recent results from GBP, either when developing products based on oligosaccharins or by applying and extending it to production scale, demonstrate the potential introduction of some of these products to benefit Cuban and international agriculture, such as *in vitro* culture hormone substitutes, protection alternatives against stress and crop growth and yield regulators.

ACKNOWLEDGEMENTS

The authors wish to thank the International Foundation for Science (IFS) and the Organization for the Prohibition of Chemical Weapons (OPCW) for its contribution to researches that led to INCA's Group of Bioactive Products' results set out in this review. The support of this organization was given through F-4446-1 and F-4446-2F projects, by means of funding for equipment, reagents, supplies and scientific information.

BIBLIOGRAPHY

1. Agrios, G. N. ''How pathogens attack plants''. En: *Plant Pathology*, edit. Academic Press, 25 de enero de 2005, pp. 37-82, ISBN 978-0-08-047378-9.
2. Agrios, G. N. ''How plants defend themselves against pathogens''. En: *Plant Pathology*, edit. Academic Press, 25 de enero de 2005, pp. 107-161, ISBN 978-0-08-047378-9.
3. Ebel, J. y Mithöfer, A. ''Early events in the elicitation of plant defence''. *Planta*, vol. 206, no. 3, agosto de 1998, pp. 335-348, ISSN 0032-0935, 1432-2048, DOI 10.1007/s004250050409.
4. Esquerré, T. M. T.; Boudart, G. y Dumas, B. ''Cell wall degrading enzymes, inhibitory proteins, and oligosaccharides participate in the molecular dialogue between plants and pathogens''. *Plant Physiology and Biochemistry*, vol. 38, no. 1-2, enero de 2000, pp. 157-163, ISSN 0981-9428, DOI 10.1016/S0981-9428(00)00161-3.
5. Ridley, B. L.; O'Neill, M. A. y Mohnen, D. ''Pectins: structure, biosynthesis, and oligogalacturonide-related signaling''. *Phytochemistry*, vol. 57, no. 6, julio de 2001, pp. 929-967, ISSN 0031-9422, DOI 10.1016/S0031-9422(01)00113-3.
6. Côté, F.; Ham, K. S.; Hahn, M. G. y Bergmann, C. W. ''Oligosaccharide Elicitors in Host-Pathogen Interactions'' [en línea]. En: *Plant-Microbe Interactions*, edit. Springer US, 1998, pp. 385-432, ISBN 978-1-4899-1709-6, [Consultado: 1 de diciembre de 2015], Disponible en: <http://link.springer.com/pter/10.1007/978-1-4899-1707-2_13>.
7. Shibuya, N. y Minami, E. ''Oligosaccharide signalling for defence responses in plant''. *Physiological and Molecular Plant Pathology*, vol. 59, no. 5, noviembre de 2001, pp. 223-233, ISSN 0885-5765, DOI 10.1006/pmpp.2001.0364.

8. Hayashi, T. ''Xyloglucans in the Primary Cell Wall''. *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 40, no. 1, 1989, pp. 139-168, ISSN 0066-4294, DOI 10.1146/annurev.pp.40.060189.001035.
9. Cutillas, I. A.; Fulton, D. C.; Fry, S. C. y Lorences, E. P. ''Xyloglucan-derived oligosaccharides induce ethylene synthesis in persimmon (*Diospyros kaki* L.) fruit''. *Journal of Experimental Botany*, vol. 49, no. 321, 4 de enero de 1998, pp. 701-706, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/49.321.701.
10. Marry, M.; Cavalier, D. M.; Schnurr, J. K.; Netland, J.; Yang, Z.; Pezeshk, V.; York, W. S.; Pauly, M. y White, A. R. ''Structural characterization of chemically and enzymatically derived standard oligosaccharides isolated from partially purified tamarind xyloglucan''. *Carbohydrate Polymers*, vol. 51, no. 3, 15 de febrero de 2003, pp. 347-356, ISSN 0144-8617, DOI 10.1016/S0144-8617(02)00189-3.
11. Cutillas, I. A. y Lorences, E. P. ''Effect of Xyloglucan Oligosaccharides on Growth, Viscoelastic Properties, and Long-Term Extension of Pea Shoots''. *Plant Physiology*, vol. 113, no. 1, 1 de enero de 1997, pp. 103-109, ISSN 1532-2548, DOI 10.1104/pp.113.1.103.
12. Kaku, T.; Tabuchi, A.; Wakabayashi, K.; Kamisaka, S. y Hoson, T. ''Action of Xyloglucan Hydrolase within the Native Cell Wall Architecture and Its Effect on Cell Wall Extensibility in Azuki Bean Epicotyls''. *Plant and Cell Physiology*, vol. 43, no. 1, 15 de enero de 2002, pp. 21-26, ISSN 0032-0781, 1471-9053, DOI 10.1093/pcp/pcf004.
13. Spaink, H. P. ''Root Nodulation and Infection Factors Produced by Rhizobial Bacteria''. *Annual Review of Microbiology*, vol. 54, no. 1, 2000, pp. 257-288, ISSN 0066-4227, 1545-3251, DOI 10.1146/annurev.micro.54.1.257.
14. Badawy, M. E. I. y Rabea, E. I. ''A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection''. *International Journal of Carbohydrate Chemistry*, vol. 2011, 19 de junio de 2011, p. 29, ISSN 1687-9341, DOI 10.1155/2011/460381.
15. Harish, P. K. V. y Tharanathan, R. N. ''Chitin/chitosan: modifications and their unlimited application potential-an overview''. *Trends in Food Science & Technology*, vol. 18, no. 3, marzo de 2007, pp. 117-131, ISSN 0924-2244, DOI 10.1016/j.tifs.2006.10.022.
16. Falcón, R. A.; Rodríguez, A. T.; Ramírez, M. A.; Rivero, D.; Martínez, B.; Cabrera, J. C.; Costales, D.; Cruz, A.; González, L. G.; Jiménez, M. C.; Jiménez, L.; Hernández, I.; Gonzáles, P. D. y Márquez, R. ''Chitosans as bioactive macromolecules to protect conomically relevant crops from their main pathogens''. *Biotecnología Aplicada*, vol. 27, no. 4, diciembre de 2010, pp. 305-309, ISSN 1027-2852.
17. Marfà, V.; Gollin, D. J.; Eberhard, S.; Mohnen, D.; Albersheim, P. ''Oligogalacturonides are able to induce flowers to form on tobacco explants''. *The Plant Journal*, vol. 1, no. 2, 1991, pp. 217–225, ISSN 1365-313X, 0960-7412, DOI http://dx.doi.org/10.1111/j.1365-313X.1991.00217.x.
18. Prithiviraj, B.; Souleimanov, A.; Zhou, X. y Smith, D. L. ''Differential response of soybean (*Glycine max* (L.) Merr.) genotypes to lipo-chito-oligosaccharide Nod Bj V (C(18:1) MeFuc)''. *Journal of Experimental Botany*, vol. 51, no. 353, diciembre de 2000, pp. 2045-2051, ISSN 0022-0957, DOI http://dx.doi.org/10.1093/jexbot/51.353.2045.
19. Gage, D. J. ''Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes''. *Microbiology and molecular biology reviews: MMBR*, vol. 68, no. 2, junio de 2004, pp. 280-300, ISSN 1092-2172, DOI 10.1128/MMBR.68.2.280-300.2004.
20. Chibu, H.; Shibayama, H. y Arima, S. ''Effects of Chitosan Application on the Shoot Growth of Rice and Soybean''. *Japanese journal of crop science*, vol. 71, no. 2, 2002, pp. 206-211, ISSN 1349-0990, 0011-1848, DOI 10.1626/jcs.71.206.
21. Cid, M.; González, O. J. L.; Lezcano, Y. y Nieves, N. ''Influencia del Pectimorf sobre la calidad de la semilla artificial de caña de azúcar (*Saccharum* spp)''. *Cultivos Tropicales*, vol. 27, no. 1, 2006, pp. 31–34, ISSN 0258-5936.
22. Nieves, N. ''Evaluación del Pectimorf como complemento del 2,4-D en el proceso de embriogénesis somática de caña de azúcar (*Saccharum* sp.)''. *Cultivos Tropicales*, vol. 27, no. 1, 2013, pp. 25–30, ISSN 0258-5936.
23. Izquierdo, H.; Núñez, M.; González, M. C.; Proenza, R. y Cabrera, J. C. ''Influencia de un oligogalacturónido en la aclimatización de vitroplantas de banano (*Musa* spp.) del clon FHIA-18 (AAAB)''. *Cultivos Tropicales*, vol. 30, no. 1, marzo de 2009, pp. 00-00, ISSN 0258-5936.
24. Ochoa, V. M.; Vargas, A. I.; Islas, M. A.; González, A. G. y Martínez, T. M. Á. ''Pectin-derived oligosaccharides increase color and anthocyanin content in Flame Seedless grapes''. *Journal of the Science of Food and Agriculture*, vol. 91, no. 10, 15 de agosto de 2011, pp. 1928-1930, ISSN 1097-0010, DOI 10.1002/jsfa.4412.
25. Benítez, B.; Soto, F.; Yong, A. y Núñez, M. ''Crecimiento de plantas de palma areca (*Dypsis lutescens*, H. Wendel) con aspersiones foliares de una mezcla de oligogalacturónidos''. *Cultivos Tropicales*, vol. 29, no. 3, septiembre de 2008, pp. 81-85, ISSN 0258-5936.
26. Alvarez, B. I.; Reynaldo, E. I.; Cartaya, R. O. y Teheran, Z. ''Efectos de una mezcla de oligogalacturónidos en la morfología de hortalizas de importancia económica''. *Cultivos Tropicales*, vol. 32, no. 3, septiembre de 2011, pp. 52-57, ISSN 0258-5936.
27. Terry, A. E.; Ruiz, P. J.; Tejeda, P. T.; Reynaldo, E. I. y Díaz, de A. M. M. ''Respuesta del cultivo de la lechuga (*Lactuca sativa* L.) a la aplicación de diferentes productos bioactivos''. *Cultivos Tropicales*, vol. 32, no. 1, 2011, pp. 28-37, ISSN 0258-5936.

28. Spiro, M. D.; Bowers, J. F. y Cosgrove, D. J. ''A Comparison of Oligogalacturonide- and Auxin-Induced Extracellular Alkalinization and Growth Responses in Roots of Intact Cucumber Seedlings''. *Plant Physiology*, vol. 130, no. 2, 10 de enero de 2002, pp. 895-903, ISSN 1532-2548, DOI 10.1104/pp.006064.
29. Plana, D. "Actividad biológica del Pectimorf en la morfogénesis *in vitro* del tomate (*Lycopersicon esculentum*, Mill) var. Amalia''. *Cultivos Tropicales*, vol. 24, no. 1, 2003, pp. 29–33, ISSN 0258-5936.
30. Falcón, A. B. ''Actividad enraizadora de una mezcla de oligogalacturónidos en pecíolos de violeta africana''. *Cultivos Tropicales*, vol. 28, no. 2, 2012, pp. 87–90, ISSN 0258-5936.
31. McDougall, G. J. y Fry, S. C. ''Xyloglucan Oligosaccharides Promote Growth and Activate Cellulase: Evidence for a Role of Cellulase in Cell Expansion''. *Plant Physiology*, vol. 93, no. 3, 7 de enero de 1990, pp. 1042-1048, ISSN 1532-2548, DOI 10.1104/pp.93.3.1042.
32. Vargas, R. C.; Reicher, F.; Sierakowski, M. R.; Heyraud, A.; Driguez, H. y Liénart, Y. "Xyloglucan Octasaccharide XXLGol Derived from the Seeds of *Hymenaea courbaril* Acts as a Signaling Molecule". *Plant Physiology*, vol. 116, no. 3, 3 de enero de 1998, pp. 1013-1021, ISSN 1532-2548, DOI 10.1104/pp.116.3.1013.
33. Acosta, A.; Rocha, M. y González, P. L. "Molecular and morphological evidences of the effect of xyloglucans on stress-response in *Arabidopsis thaliana*". En: *Memorias del IV Simposio Internacional en Bioquímica y Biología Molecular*, edit. Centro internacional de conferencias de La Habana, La Habana, Cuba, 2009.
34. Cabrera, J. C.; Wégria, G.; Onderwater, R. C. A.; González, G.; Nápoles, M. C.; Falcón, R. A. B.; Costales, D.; Rogers, H. J.; Diosdado, E.; González, S.; Cabrera, G.; González, L. y Wattiez, R. ''Practical use of oligosaccharins in agriculture". *Acta Horticulturae*, no. 1009, octubre de 2013, pp. 195-212, ISSN 0567-7572, 2406-6168, DOI 10.17660/ActaHortic.2013.1009.24.
35. González, P. L.; Vázquez, G. A.; Perrotta, L.; Acosta, A.; Scriven, S. A.; Herbert, R.; Cabrera, J. C.; Francis, D. y Rogers, H. J. "Oligosaccharins and Pectimorf® stimulate root elongation and shorten the cell cycle in higher plants". *Plant Growth Regulation*, vol. 68, no. 2, 3 de mayo de 2012, pp. 211-221, ISSN 0167-6903, 1573-5087, DOI 10.1007/s10725-012-9709-z.
36. Hadwiger, L. A. *Method for treating cereal crop seed with chitosan to enhance yield, root growth and stem strength* [en línea]. no. US 4886541 A, Inst. Washington State University Research Foundation, 12 de diciembre de 1989, [Consultado: 1 de diciembre de 2015], Disponible en: <http://www.google.com/patents/US4886541>.
37. Utsunomiya, N.; Kinai, H.; Matsui, Y. y Takebayashi, T. "The Effects of Chitosan Oligosaccharides Soil Conditioner and Nitrogen Fertilizer on the Flowering and Fruit Growth of Purple Passionfruit (*Passiflora edulis* Sims var. edulis)". *Journal of the Japanese Society for Horticultural Science*, vol. 67, no. 4, 1998, pp. 567-571, ISSN 1882-336X, 1882-3351, DOI 10.2503/jjshs.67.567.
38. Ohta, K.; Morishita, S.; Suda, K.; Kobayashi, N. y Hosoki, T. "Effects of Chitosan Soil Mixture Treatment in the Seedling Stage on the Growth and Flowering of Several Ornamental Plants". *Journal of the Japanese Society for Horticultural Science*, vol. 73, no. 1, 2004, pp. 66-68, ISSN 1882-336X, 1882-3351, DOI 10.2503/jjshs.73.66.
39. Freepons, D. E. *Plant growth regulators derived from chitin* [en línea]. no. US4964894 A, 23 de octubre de 1990, [Consultado: 1 de diciembre de 2015], Disponible en: <http://www.google.com.cu/patents/US4964894>.
40. Cho, M. H.; No, H. K. y Prinyawiwatkul, W. ''Chitosan Treatments Affect Growth and Selected Quality of Sunflower Sprouts''. *Journal of Food Science*, vol. 73, no. 1, 1 de enero de 2008, pp. S70-S77, ISSN 1750-3841, DOI 10.1111/j.1750-3841.2007.00607.x.
41. Sharathchandra, R. G.; Raj, S. N.; Shetty, N. P.; Amruthesh, K. N. y Shetty, H. S. ''A Chitosan formulation Elexa™ induces downy mildew disease resistance and growth promotion in pearl millet". *Crop Protection*, vol. 23, no. 10, octubre de 2004, pp. 881-888, ISSN 0261-2194, DOI 10.1016/j.cropro.2003.12.008.
42. Bhaskara, R. M. V.; Arul, J.; Angers, P. y Couture, L. "Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality". *Journal of Agricultural and Food Chemistry*, vol. 47, no. 3, 1999, pp. 1208–1216, ISSN 0021-8561, DOI http://dx.doi.org/10.1021/jf981225k.
43. Shao, C. X.; Hu, J.; Song, W. J. y Hu, W. M. "Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling". *Journal of Zhejiang University (Agriculture and Life Science)*, vol. 31, no. 6, 2005, pp. 705–708, ISSN 1008-9209.
44. Zhou, Y. G.; Yang, Y. D.; Qi, Y. G.; Zhang, Z. M.; Wang, X. J. y Hu, X. J. "Effects of chitosan on some physiological activity in germinating seed of peanut". *Journal of Peanut Science*, vol. 31, no. 1, 2002, pp. 22–25, ISSN 0095-3679.
45. Boonlertnirun, S.; Boonraung, C. y Suvanasara, R. ''Application of chitosan in rice production''. *Journal of Metals, Materials and Minerals*, vol. 18, no. 2, 2008, pp. 47–52, ISSN 0857-6149.
46. Dzung, N. ''Enhancing Crop Production with Chitosan and Its Derivatives'' [en línea]. En: *Chitin, Chitosan, Oligosaccharides and Their Derivatives*, edit. CRC Press, 14 de julio de 2010, pp. 619-631, ISBN 978-1-4398-1603-5, [Consultado: 1 de diciembre de 2015], Disponible en: <http://www.crcnetbase.com/doi/abs/10.1201/EBK1439816035-c42>.

47. Khan, W. M.; Prithiviraj, B. y Smith, D. L. ''Effect of Foliar Application of Chitin and Chitosan Oligosaccharides on Photosynthesis of Maize and Soybean''. *Photosynthetica*, vol. 40, no. 4, diciembre de 2002, pp. 621-624, ISSN 0300-3604, 1573-9058, DOI 10.1023/A:1024320606812.
48. Abdel, M. A. M. R.; Tantawy, A. S.; El-Nemr, M. A. y Sassine, Y. N. ''Growth and yield responses of strawberry plants to chitosan application''. *European Journal of Scientific Research*, vol. 39, no. 1, 2010, pp. 161-168, ISSN 1450-216X, 1450-202X.
49. Chibu, H. y Shibayama, H. ''Effects of chitosan application on the growth of several crops''. En: Uragami T., *Chitin and Chitosan in Life Science: Proceedings of the Eighth International Chitin and Chitosan Conference and Fourth Asia Pacific Chitin and Chitosan Symposium, Yamaguchi, Japan, September 21-23, 2000*, edit. Kodansha Scientific, 2001, pp. 235–239, ISBN 978-4-906464-13-5.
50. Kowalski, B.; Terry, F. J.; Herrera, L. y Peñalver, D. A. ''Application of Soluble Chitosan *in vitro* and in the Greenhouse to Increase Yield and Seed Quality of Potato Minitubers''. *Potato Research*, vol. 49, no. 3, 13 de febrero de 2007, pp. 167-176, ISSN 0014-3065, 1871-4528, DOI 10.1007/s11540-006-9015-0.
51. Lee, S.; Choi, H.; Suh, S.; Doo, I. S.; Oh, K. Y.; Choi, E. J.; Taylor, A. T. S.; Low, P. S. y Lee, Y. ''Oligogalacturonic Acid and Chitosan Reduce Stomatal Aperture by Inducing the Evolution of Reactive Oxygen Species from Guard Cells of Tomato and *Commelina communis*''. *Plant Physiology*, vol. 121, no. 1, 9 de enero de 1999, pp. 147-152, ISSN 1532-2548, DOI 10.1104/pp.121.1.147.
52. Iriti, M.; Picchi, V.; Rossoni, M.; Gomarasca, S.; Ludwig, N.; Gargano, M. y Faoro, F. ''Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure''. *Environmental and Experimental Botany*, vol. 66, no. 3, septiembre de 2009, pp. 493-500, ISSN 0098-8472, DOI 10.1016/j.envexpbot.2009.01.004.
53. Bittelli, M.; Flury, M.; Campbell, G. S. y Nichols, E. J. ''Reduction of transpiration through foliar application of chitosan''. *Agricultural and Forest Meteorology*, vol. 107, no. 3, 2 de abril de 2001, pp. 167-175, ISSN 0168-1923, DOI 10.1016/S0168-1923(00)00242-2.
54. Lhuissier, F. G. P.; Ruijter, N. C. A. D.; Sieberer, B. J.; Esseling, J. J. y Emons, A. M. C. ''Time Course of Cell Biological Events Evoked in Legume Root Hairs by Rhizobium Nod Factors: State of the Art''. *Annals of Botany*, vol. 87, no. 3, 3 de enero de 2001, pp. 289-302, ISSN 0305-7364, 1095-8290, DOI 10.1006/anbo.2000.1333.
55. Prithiviraj, B.; Zhou, X.; Souleimanov, A.; Khan, W. M.; Smith, D. L. y Kahn, W. M. ''A host-specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop plants''. *Planta*, vol. 216, no. 3, enero de 2003, pp. 437-445, ISSN 0032-0935, DOI 10.1007/s00425-002-0928-9.
56. D'Haeze, W. y Holsters, M. ''Nod factor structures, responses, and perception during initiation of nodule development''. *Glycobiology*, vol. 12, no. 6, 6 de enero de 2002, p. 79R-105R, ISSN 0959-6658, 1460-2423, DOI 10.1093/glycob/12.6.79R.
57. Costales, D. ''Efecto de derivados de quitosana en la simbiosis *Bradyrhizobium*-soya''. *Cultivos Tropicales*, vol. 26, no. 1, 2013, pp. 83-87, ISSN 0258-5936.
58. Costales, D.; Nápoles, M. y Falcón, A. ''Influencia de oligosacáridos de quitosana y pectina en la interacción simbiótica Soya-*Bradyrhizobium*''. *Revista Cubana de Ciencia Agrícola*, vol. 41, no. 2, 2007, pp. 175–181, ISSN 0034-7485, 2079-3472.
59. Sharp, J. K.; McNeil, M. y Albersheim, P. ''The primary structures of one elicitor-active and seven elicitor-inactive hexa(beta-D-glucopyranosyl)-D-glucitols isolated from the mycelial walls of *Phytophthora megasperma* f. sp. glycinea''. *The Journal of Biological Chemistry*, vol. 259, no. 18, 25 de septiembre de 1984, pp. 11321-11336, ISSN 0021-9258.
60. Cosio, E. G.; Feger, M.; Miller, C. J.; Antelo, L. y Ebel, J. ''High-affinity binding of fungal β-glucan elicitors to cell membranes of species of the plant family Fabaceae''. *Planta*, vol. 200, no. 1, septiembre de 1996, pp. 92-99, ISSN 0032-0935, 1432-2048, DOI 10.1007/BF00196654.
61. Klarzynski, O.; Plesse, B.; Joubert, J.-M.; Yvin, J.-C.; Kopp, M.; Kloareg, B. y Fritig, B. ''Linear β-1,3 Glucans Are Elicitors of Defense Responses in Tobacco''. *Plant Physiology*, vol. 124, no. 3, 11 de enero de 2000, pp. 1027-1038, ISSN 1532-2548, DOI 10.1104/pp.124.3.1027.
62. Aziz, A.; Poinssot, B.; Daire, X.; Adrian, M.; Bézier, A.; Lambert, B.; Joubert, J. M. y Pugin, A. ''Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*''. *Molecular plant-microbe interactions: MPMI*, vol. 16, no. 12, diciembre de 2003, pp. 1118-1128, ISSN 0894-0282, DOI 10.1094/MPMI.2003.16.12.1118.
63. Aziz, A.; Heyraud, A. y Lambert, B. ''Oligogalacturonide signal transduction, induction of defense-related responses and protection of grapevine against *Botrytis cinerea*''. *Planta*, vol. 218, no. 5, 14 de noviembre de 2003, pp. 767-774, ISSN 0032-0935, 1432-2048, DOI 10.1007/s00425-003-1153-x.
64. Bartnicki, G. S. y Wang, M. C. ''Biochemical aspects of morphogenesis in Phytophthora''. En: Erwin D. C., Bartnicki G. S., Tsao P. H., y Society A. P., *Phytophthora: its biology, taxonomy, ecology, and pathology*, edit. American Phytopathological Society, 1983, pp. 121-138, ISBN 978-0-89054-050-3.
65. Nishizawa, Y.; Kawakami, A.; Hibi, T.; He, D. Y.; Shibuya, N. y Minami, E. ''Regulation of the chitinase gene expression in suspension-cultured rice cells by N-acetylchitooligosaccharides: differences in the signal transduction pathways leading to the activation of elicitor-responsive genes''. *Plant Molecular Biology*, vol. 39, no. 5, marzo de 1999, pp. 907-914, ISSN 0167-4412, 1573-5028, DOI 10.1023/A:1006161802334.

66. Zhao, J.; Davis, L. C. y Verpoorte, R. ''Elicitor signal transduction leading to production of plant secondary metabolites''. *Biotechnology Advances*, vol. 23, no. 4, junio de 2005, pp. 283-333, ISSN 0734-9750, DOI 10.1016/j.biotechadv.2005.01.003.
67. Bautista, B. S.; Hernández, L. A. N.; Velázquez, del V. M. G.; Hernández, L. M.; Ait Barka, E.; Bosquez, M. E. y Wilson, C. L. ''Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities''. *Crop Protection*, vol. 25, no. 2, febrero de 2006, pp. 108-118, ISSN 0261-2194, DOI 10.1016/j.cropro.2005.03.010.
68. Khan, W.; Prithiviraj, B. y Smith, D. L. "Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves''. *Journal of Plant Physiology*, vol. 160, no. 8, 2003, pp. 859-863, ISSN 0176-1617, DOI 10.1078/0176-1617-00905.
69. El Hadrami, A.; Adam, L. R.; El Hadrami, I. y Daayf, F. "Chitosan in Plant Protection''. *Marine Drugs*, vol. 8, no. 4, 30 de marzo de 2010, pp. 968-987, ISSN 1660-3397, DOI 10.3390/md8040968.
70. Falcón, R. A. B.; Costales, D.; Cabrera, J. C. y Martínez, T. M. Á. ''Chitosan physico–chemical properties modulate defense responses and resistance in tobacco plants against the oomycete *Phytophthora nicotianae*". *Pesticide Biochemistry and Physiology*, vol. 100, no. 3, julio de 2011, pp. 221-228, ISSN 0048-3575, DOI 10.1016/j.pestbp.2011.04.005.
71. Xu, J.; Zhao, X.; Han, X. y Du, Y. "Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*''. *Pesticide Biochemistry and Physiology*, vol. 87, no. 3, marzo de 2007, pp. 220-228, ISSN 0048-3575, DOI 10.1016/j.pestbp.2006.07.013.
72. Xu, J.; Zhao, X.; Wang, X.; Zhao, Z. y Du, Y. "Oligochitosan inhibits *Phytophthora capsici* by penetrating the cell membrane and putative binding to intracellular targets''. *Pesticide Biochemistry and Physiology*, vol. 88, no. 2, junio de 2007, pp. 167-175, ISSN 0048-3575, DOI 10.1016/j.pestbp.2006.10.010.
73. Falcón, A. B.; Cabrera, J. C.; Costales, D.; Ramírez, M. A.; Cabrera, G.; Toledo, V. y Martínez, T. M. A. "The effect of size and acetylation degree of chitosan derivatives on tobacco plant protection against *Phytophthora parasitica nicotianae*''. *World Journal of Microbiology and Biotechnology*, vol. 24, no. 1, 23 de junio de 2007, pp. 103-112, ISSN 0959-3993, 1573-0972, DOI 10.1007/s11274-007-9445-0.
74. Rodríguez, A. B. F.; Menéndez, D. C.; Delgado, E. O.; Díaz, O. L. y Pino, J. C. C. "Evaluation of chitosan as an inhibitor of soil-borne pathogens and as an elicitor of defence markers and resistance in tobacco plants". *Spanish Journal of Agricultural Research*, vol. 5, no. 4, 1 de diciembre de 2007, pp. 533-541, ISSN 2171-9292, DOI 10.5424/sjar/2007054-274.
75. Palma, G. J.; Jansson, H.-B.; Salinas, J. y Lopez, L. L. V. "Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi". *Journal of Applied Microbiology*, vol. 104, no. 2, febrero de 2008, pp. 541-553, ISSN 1365-2672, DOI 10.1111/j.1365-2672.2007.03567.x.
76. Hernández, L. A. N.; Hernández, M. M.; Velázquez, del V. M. G.; Guerra, S. M. G. y Melo, G. G. E. "Actividad antifúngica del quitosano en el control de *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. y *Mucor* spp". *Revista Mexicana de Fitopatología*, vol. 25, no. 2, 2007, pp. 109–113, ISSN 0185-3309, 2007-8080.
77. Liu, J.; Tian, S.; Meng, X. y Xu, Y. ''Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit''. *Postharvest Biology and Technology*, vol. 44, no. 3, junio de 2007, pp. 300-306, ISSN 0925-5214, DOI 10.1016/j.postharvbio.2006.12.019.
78. Bhaskara, R. M.; Arul, J.; Ait-Barka, E.; Angers, P.; Richard, C. y Castaigne, F. ''EVect of Chitosan on Growth and Toxin Production by *Alternaria alternata* f. sp. lycopersici''. *Biocontrol Science and Technology*, vol. 8, no. 1, 1 de marzo de 1998, pp. 33-43, ISSN 0958-3157, DOI 10.1080/09583159830414.
79. Plascencia, J. M.; Viniegra, G.; Olayo, R.; Castillo, O. M. M. y Shirai, K. ''Effect of Chitosan and Temperature on Spore Germination of *Aspergillus niger*". *Macromolecular Bioscience*, vol. 3, no. 10, 1 de octubre de 2003, pp. 582-586, ISSN 1616-5195, DOI 10.1002/mabi.200350024.
80. Sánchez, D. D.; Bautista, B. S. y Castillo, P. "Efecto del quitosano en el desarrollo y morfología de *Alternaria alternata* (Fr.) Keissl". *Anales de Biología*, vol. 29, 2007, pp. 23–32, ISSN 1138-3399. 1989-2128.
81. Ghaouth, A. E. "Effect of Chitosan on Cucumber Plants: Suppression of *Pythium aphanidermatum* and Induction of Defense Reactions". *Phytopathology*, vol. 84, no. 3, 1994, p. 313, ISSN 0031949X, DOI 10.1094/Phyto-84-313.
82. Barka, E. A.; Eullaffroy, P.; Clément, C. y Vernet, G. "Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*". *Plant Cell Reports*, vol. 22, no. 8, 1 de noviembre de 2003, pp. 608-614, ISSN 0721-7714, 1432-203X, DOI 10.1007/s00299-003-0733-3.
83. Agostini, J. P.; Bushong, P. M. y Timmer, L. W. ''Greenhouse Evaluation of Products That Induce Host Resistance for Control of Scab, Melanose, and Alternaria Brown Spot of Citrus''. *Plant Disease*, vol. 87, no. 1, 1 de enero de 2003, pp. 69-74, ISSN 0191-2917, DOI 10.1094/PDIS.2003.87.1.69.
84. Copping, L. G. *The biopesticide manual: world compendium*. edit. British crop protection council, 1998, 333 p., ISBN 1-901396-26-6, CABDirect2.

85. Bautista, B. S.; Hernández, L. M.; Bosquez, M. E. y Wilson, C. L. ''Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit''. *Crop Protection*, vol. 22, no. 9, noviembre de 2003, pp. 1087-1092, ISSN 0261-2194, DOI 10.1016/S0261-2194(03)00117-0.
86. Jitareerat, P.; Paumchai, S.; Kanlayanarat, S. y Sangchote, S. "Effect of chitosan on ripening, enzymatic activity, and disease development in mango (*Mangifera indica*) fruit''. *New Zealand journal of crop and horticultural science*, vol. 35, no. 2, 2007, pp. 211–218, ISSN 0114-0671, 1175-8783, DOI http://dx.doi.org/10.1080/01140670709510187.
87. Chien, P. J. y Chou, C. C. ''Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of Tankan citrus fruit (*Citrus tankan* Hayata)''. *Journal of the Science of Food and Agriculture*, vol. 86, no. 12, 1 de septiembre de 2006, pp. 1964-1969, ISSN 1097-0010, DOI 10.1002/jsfa.2570.
88. Hernández, M. P.; Almenar, E.; Valle, V. D.; Velez, D. y Gavara, R. "Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria × ananassa*) quality during refrigerated storage". *Food Chemistry*, vol. 110, no. 2, 15 de septiembre de 2008, pp. 428-435, ISSN 0308-8146, DOI 10.1016/j.foodchem.2008.02.020.
89. Lin, W.; Hu, X.; Zhang, W.; John Rogers, W. y Cai, W. ''Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice". *Journal of Plant Physiology*, vol. 162, no. 8, 23 de agosto de 2005, pp. 937-944, ISSN 0176-1617, DOI 10.1016/j.jplph.2004.10.003.
90. Rodríguez, A. T.; Ramírez, M. A.; Cárdenas, R. M.; Hernández, A. N.; Velázquez, M. G. y Bautista, S. ''Induction of defense response of *Oryza sativa* L. against *Pyricularia grisea* (Cooke) Sacc. by treating seeds with chitosan and hydrolyzed chitosan''. *Pesticide Biochemistry and Physiology*, vol. 89, no. 3, noviembre de 2007, pp. 206-215, ISSN 0048-3575, DOI 10.1016/j.pestbp.2007.06.007.
91. Romanazzi, G.; Nigro, F.; Ippolito, A.; DiVenere, D. y Salerno, M. "Effects of Pre- and Postharvest Chitosan Treatments to Control Storage Grey Mold of Table Grapes". *Journal of Food Science*, vol. 67, no. 5, 1 de junio de 2002, pp. 1862-1867, ISSN 1750-3841, DOI 10.1111/j.1365-2621.2002.tb08737.x.
92. Trotel, A. P.; Couderchet, M.; Vernet, G. y Aziz, A. ''Chitosan Stimulates Defense Reactions in Grapevine Leaves and Inhibits Development of *Botrytis Cinerea*''. *European Journal of Plant Pathology*, vol. 114, no. 4, abril de 2006, pp. 405-413, ISSN 0929-1873, 1573-8469, DOI 10.1007/s10658-006-0005-5.
93. Ben, S. N.; Ardi, R.; Pinto, R.; Aki, C. y Fallik, E. ''Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan''. *Crop Protection*, vol. 22, no. 2, marzo de 2003, pp. 285-290, ISSN 0261-2194, DOI 10.1016/S0261-2194(02)00149-7.
94. Molloy, C.; Cheah, L. H. y Koolaard, J. P. "Induced resistance against *Sclerotinia sclerotiorum* in carrots treated with enzymatically hydrolysed chitosan". *Postharvest Biology and Technology*, vol. 33, no. 1, julio de 2004, pp. 61-65, ISSN 0925-5214, DOI 10.1016/j.postharvbio.2004.01.009.
95. Benhamou, N. ''Induction of Systemic Resistance to Fusarium Crown and Root Rot in Tomato Plants by Seed Treatment with Chitosan''. *Phytopathology*, vol. 84, no. 12, 1994, p. 1432, ISSN 0031949X, DOI 10.1094/Phyto-84-1432.
96. Baños, S. B. y Luna, L. B. ''Evaluación del quitosano en el desarrollo de la pudrición blanda del tomate durante el almacenamiento''. *Revista Iberoamericana de Tecnología Postcosecha*, vol. 6, no. 1, 2004, pp. 63–67, ISSN 1665-0204.
97. Coqueiro, D. S. O.; Maraschin, M. y Piero, R. M. D. ''Chitosan Reduces Bacterial Spot Severity and Acts in Phenylpropanoid Metabolism in Tomato Plants''. *Journal of Phytopathology*, vol. 159, no. 7-8, 1 de agosto de 2011, pp. 488-494, ISSN 1439-0434, DOI 10.1111/j.1439-0434.2011.01791.x.
98. Du, J.; Gemma, H. y Iwahori, S. ''Effects of Chitosan Coating on the Storage of Peach, Japanese Pear, and Kiwifruit.''. *Journal of the Japanese Society for Horticultural Science*, vol. 66, no. 1, 1997, pp. 15–22, ISSN 1880-358X, DOI http://dx.doi.org/10.2503/jjshs.66.15.
99. Zhao, X.; She, X.; Du, Y. y Liang, X. ''Induction of antiviral resistance and stimulary effect by oligochitosan in tobacco''. *Pesticide Biochemistry and Physiology*, vol. 87, no. 1, enero de 2007, pp. 78-84, ISSN 0048-3575, DOI 10.1016/j.pestbp.2006.06.006.
100. Iriti, M. y Faoro, F. "Abscisic acid is involved in chitosan-induced resistance to tobacco necrosis virus (TNV)''. *Plant Physiology and Biochemistry*, vol. 46, no. 12, diciembre de 2008, pp. 1106-1111, ISSN 0981-9428, DOI 10.1016/j.plaphy.2008.08.002.
101. Salvador, P. y Lasserre, T. *Process for increasing plants resistance to an abiotic stress* [en línea]. no. US20100304975 A1, Inst. Elicityl, 2 de diciembre de 2010, [Consultado: 1 de diciembre de 2015], Disponible en: <http://www.google.com.cu/patents/US20100304975>.
102. Xuan, T. L.; Nagasawa, N.; Matsuhashi, S.; Ishioka, N. S.; Ito, T. y Kume, T. "Effect of radiation-degraded chitosan on plants stressed with vanadium". *Radiation Physics and Chemistry*, vol. 61, no. 2, mayo de 2001, pp. 171-175, ISSN 0969-806X, DOI 10.1016/S0969-806X(00)00388-1.
103. Ortega, O.; Benavides, M. A.; Flores, O. A. y Ledezma, P. A. ''Use of the Interpolyelectrolyte Complexes of Poly (acrylic acid)-Chitosan as Inductors of Tolerance Against Pathogenic Fungi in Tomato (*Lycopersicon esculentum* Mill. var. Floradade)''. *Macromolecular Bioscience*, vol. 3, no. 10, 1 de octubre de 2003, pp. 566-570, ISSN 1616-5195, DOI 10.1002/mabi.200300021.
104. Boonlertnirun, S.; Sarobol, E. D.; Meechoui, S. y Sooksathan, I. ''Drought recovery and grain yield potential of rice after chitosan application''. *Kasetsart Journal: Natural Science*, vol. 41, 2007, pp. 1-6, ISSN 0075-5192.

105. Górnik, K.; Grzesik, M. y Romanowska, D. B. "The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress''. *Journal of Fruit and Ornamental Plant Research*, vol. 16, 2008, pp. 333–343, ISSN 1231-0948.
106. Lizárraga, P. E. G.; Torres, P. I.; Moreno, M. E. y Miranda, C. S. P. ''Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level''. *African Journal of Biotechnology*, vol. 10, no. 34, 2013, pp. 6439-6446, ISSN 1684-5315, DOI 10.4314/ajb.v10i34.
107. Kim, S. K. *Chitin, Chitosan, Oligosaccharides and Their Derivatives: Biological Activities and Applications*. edit. CRC Press, 14 de julio de 2010, 668 p., ISBN 978-1-4398-1604-2.
108. Corbera, G. J. y Nápoles, G. M. C. "Efecto de la inoculación conjunta *Bradyrhizobium elkanii*-hongos MA y la aplicación de un bioestimulador del crecimiento vegetal en soya (*Glycine max* (L.) Merrill), cultivar INCASOY-27''. *Cultivos Tropicales*, vol. 34, no. 2, junio de 2013, pp. 05-11, ISSN 0258-5936.
109. Hernández, L. "Efecto de una mezcla de oligogalacturónidos en el crecimiento y desarrollo del cultivo de *Anthurium andreanum*". *Cultivos tropicales*, vol. 28, no. 4, 2012, pp. 83–86, ISSN 0258-5936.
110. Suárez, L. "Efecto que ejercen las aspersiones foliares de una mezcla de oligogalacturónidos (Pectimorf) y la formulación a base de un análogo de brasinoesteroides (Biobras-16) en dos especies de orquídeas (*Cattleya leuddemanniana* y *Guarianthe skinneri*)''. *Cultivos Tropicales*, vol. 28, no. 4, 2007, pp. 87–91, ISSN 0258-5936.º
111. Cartaya, O. E.; Reynaldo, I.; Peniche, C. y Garrido, M. L. ''Empleo de polímeros naturales como alternativa para la remediación de suelos contaminados por metales pesados". *Revista internacional de contaminación ambiental*, vol. 27, no. 1, febrero de 2011, pp. 41-46, ISSN 0188-4999.
112. Rivero, D.; Cruz, A.; Martínez, B.; Rodríguez, A. T. y Ramírez, M. A. "Actividad antifúngica *in vitro* de dos quitosanas frente a *Fusarium subglutinans* (Wollenweber and Reinking), patógeno de arroz (*Oryza sativa* L.)''. *Revista de Protección Vegetal*, vol. 21, no. 2, 2006, ISSN 1010-2752, 2224-4697, [Consultado: 1 de diciembre de 2015], Disponible en: <http://agris.fao.org/agris-search/search.do?recordID=CU2009100417>.
113. Deyanira, R.; Cruz, A.; Martínez, B.; Rodríguez, A. T. y Ramírez, M. A. "Actividad antifúngica *in vitro* de las quitosanas K1 y Sigma frente a *Bipolaris oryzae* (B. de Haan) Shoem". *Revista de Protección Vegetal*, vol. 23, no. 1, abril de 2008, pp. 43-47, ISSN 1010-2752.
114. Rodríguez, P. A. T.; Ramírez, A. M. Á.; Cárdenas, T. R. M.; Falcón, R. A. y Bautista, B. S. "Efecto de la Quitosana en la Inducción de la Actividad de Enzimas Relacionadas con la Defensa y Protección de Plántulas de Arroz (*Oryza sativa* L.) contra *Pyricularia grisea* Sacc". *Revista Mexicana de Fitopatología*, vol. 24, no. 1, 2006, pp. 1-7, ISSN 0185-3309, 2007-8080.
115. González, L.; Falcan, A.; Jiménez, M. C.; Jiménez, L.; Silvente, J. y Terrero, J. C. "Evaluación de tres dosis de quitosana en el cultivo de pepino en un periodo tardío". *Revista amazónica: Ciencia y tecnología*, vol. 1, no. 2, 2012, pp. 134-139, ISSN 1390-5600.
116. Arteaga, M. C. J.; Gómez, G. G.; Rodríguez, A. F. y Quintana, O. ''Evaluación de tres bioestimulantes sobre la incidencia de plagas en el maíz (*Zea mays* L.) en la provincia de Santiago de Cuba". *Centro Agrícola*, vol. 37, no. 2, 2010, pp. 45–48, ISSN 2072-2001.
117. Nápoles, M. C. *Medio de cultivo para Bradyrhizobium japonicum. Biopreparado resultante*. no. 22 797, Inst. OCPI, Cuba, 2002.
118. Nápoles, M. C.; Luyten, E.; Dombrecht, B.; Laeremans, T.; Vanderleyden, J.; Costales, D.; Gutiérrez, A. y Corbera, J. "Growth media modulating the symbiotic efficiency of *Bradyrhizobium elkanii*''. *Symbiosis*, vol. 38, no. 1, 2005, pp. 87-98, ISSN 0334-5114, 1878-7665.
119. Nápoles, M. C.; Cabrera, J. C.; Varela, M.; González, A. G.; Nogueras, F.; Cricco, J.; Guevara, E. y Meira, S. ''Influencia de inoculantes y factores edáficos en el rendimiento de la soya''. *Cultivos Tropicales*, vol. 30, no. 3, septiembre de 2009, pp. 18-22, ISSN 0258-5936.
120. Nápoles, M. C.; Guevara, E.; Montero, F.; Rossi, A. y Ferreira, A. ''Role of *Bradyrhizobium japonicum* induced by genistein on soybean stressed by water deficit''. *Spanish Journal of Agricultural Research*, vol. 7, no. 3, 1 de septiembre de 2009, pp. 665-671, ISSN 2171-9292, DOI 10.5424/sjar/2009073-451.
121. Duzan, H. M.; Mabood, F.; Zhou, X.; Souleimanov, A. y Smith, D. L. ''Nod factor induces soybean resistance to powdery mildew''. *Plant Physiology and Biochemistry*, vol. 43, no. 10-11, octubre de 2005, pp. 1022-1030, ISSN 0981-9428, DOI 10.1016/j.plaphy.2005.08.004.

Received: December 26th, 2014
Accepted: March 20th, 2015

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas