





Review

Sustainable Agriculture Systems in Vegetable Production Using Chitin and Chitosan as Plant Biostimulants

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Abstract: Chitin and chitosan are natural compounds that are biodegradable and nontoxic and have gained noticeable attention due to their effective contribution to increased yield and agro-environmental sustainability. Several effects have been reported for chitosan application in plants. Particularly, it can be used in plant defense systems against biological and environmental stress conditions and as a plant growth promoter—it can increase stomatal conductance and reduce transpiration or be applied as a coating material in seeds. Moreover, it can be effective in promoting chitinolytic microorganisms and prolonging storage life through post-harvest treatments, or benefit nutrient delivery to plants since it may prevent leaching and improve slow release of nutrients in fertilizers. Finally, it can remediate polluted soils through the removal of cationic and anionic heavy metals and the improvement of soil properties. On the other hand, chitin also has many beneficial effects such as plant growth promotion, improved plant nutrition and ability to modulate and improve plants' resistance to abiotic and biotic stressors. The present review presents a literature overview regarding the effects of chitin, chitosan and derivatives on horticultural crops, highlighting their important role in modern sustainable crop production; the main limitations as well as the future prospects of applications of this particular biostimulant category are also presented.

Keywords: chitosan derivatives; oligochitosan; vegetable crops; abiotic stress; biotic stress



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1. Introduction

Modern agriculture needs to be adapted to the ongoing climate change and the growing food demands due to increasing population. Considering the finite natural resources, sustainable cropping is of major importance, especially in horticultural crops that are more susceptible to climate extremities and more demanding in terms of agricultural inputs [1,2]. In this context, biostimulant application is considered a novel, eco-friendly farming practice that marries two otherwise contrasting concepts, namely crop intensification and sustainability [3,4]. So far, biostimulant products form a significant part of the global farming industry, showing increasing trends over the years and in the years to come [5]. There are numerous reports regarding their positive effects on crops, especially under biotic and abiotic stress conditions [6–8], while significant research is continuously conducted to find and/or produce new biostimulatory products [9–11], as well as to reveal the mechanisms of action behind the observed effects [12–14]. However, the variability in the composition of biostimulant products, as well as the lack of common application protocols for the various products, may create inconsistencies between the observed results and complicate the efforts to reveal the actual mechanisms behind the biostimulatory effects, which may include physiological processes, morphological changes and hormonal regulation [12,14–16].

Biostimulants' beneficial activity involves the induction of root growth, the improvement in nutrients uptake and the production of phytohormones, while osmotic adjustment via synthesis of organic osmolytes has been also confirmed [17–20]. Biostimulants also can be used to reduce application of mineral inorganic fertilizers and are being considered an environmental friendly practice with no significant adverse impacts on both fruit quality and total yield [21–24]. Humic acids, fulvic acid, protein hydrolysates, seaweed extracts, N-containing compounds, botanicals, seaweed extracts, chitosan and other related biopolymers, beneficial bacteria and fungi and inorganic compounds are the main categories of plant biostimulants [25–27]. However, different classification approaches have been suggested so far, based either on the origin of each biostimulant, namely biological or non-biological, microbial and non-microbial, or on the mode of action which divides biostimulants into phytohormonal and non-phytohormonal ones [28].

Modern crop production has to cope with biotic and abiotic stressors such as soil and irrigation water salinity, water limitations, extreme and untimely weather phenomena and infections from pathogens and pests, which severely affect crop performance and quality of the final products [29,30]. In this context, the application of chitin, chitosan and derived biopolymers can play a pivotal role due to their confirmed biostimulatory activity in various crops, especially in vegetable species, which are more prone to stressors [31–33]. Different sources of chitin and chitosan in nature are Crustaceans (shrimp, lobster, king crab), Fungi (*Mucor rouxii*, *Aspergillus niger*, *Penicillium crysogenum*, *Lactarius vellereus*), Insects (lady bug, silk worm, wax worm, butterfly) and mollusks (shell oysters, squid pen) [34]. Crustacean shells are the most notable chitin source, and chitin recovery involves three steps consisting of demineralization, deproteination and elimination of pigments and lipids [35,36]. Microbial proteases such as *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Serratia marcescens*, etc. are the most notable applied strains of chitin and chitosan production [35].

Economic indicators such as return on investment, net present value and payback period have been reported as important characteristics for a mass integrated biorefinery approach to produce chitin and chitosan [36]. Considering the great amounts of chitinous waste production (e.g., 2.1–2.7 Mt in 2011), there is great economic potential of finding alternative uses of chitin and valorizing biowaste [37]. Various application have been suggested for chitin obtained mostly from crustacean shells, which are also a very good source for carotenoids recovery (e.g., astaxanthin) [38]. Due to its biological and physicochemical properties, the most important applications of chitin and its derivatives are in (a) food application, due to chitosan's ability in lowering cholesterol by blocking the absorption of cholesterol and dietary fat, which facilitates weight and body fat loss in the human body [39], controls over-nutrition and achieves insulin resistance therapy [40,41]; and (b) biomedical application, having tremendous biological benefits such as biodegradation, biocompatibility, anticancer, antibacterial, non-toxicity, immune-stimulating effects, hemostatic activity in cell culture, wound healing, tissue engineering and drug delivery [42]. Chitooligosaccharides and their derivatives are the appropriate agents capable of treating or preventing various chronic inflammation such colitis, hepatitis, gastritis, periodontal disease and through drug delivery systems [43–45]; (c) agricultural applications [46–48], and (d) bionanotechnology, such as the versatile potential uses in cosmetics, photography, ophthalmology, textile industry and water and waste treatment [49,50]. It has been also reported that large-scale chitosan commercialization originates from the chemical alkaline hydrolysis of shrimp chitin, with a cost of nearly USD 10/g (Sigma Chemical Con., St. Louis, MO 63118, USA) [51], but agro-industrial wastewaters have been also used as alternative media for fungi grown in submerged fermentation, which are readily available and have a low cost to use, saving around 38–73% of the total cost of the bioproduct production [51,52]. However, cost production is flexible since it includes transportation and labor costs, which vary significantly around the world [38].

Therefore, the present review provides an overview of the recent trends in biostimulant application focusing on the effects of chitin, chitosan and derivatives on the main vegetable

crops, as well as on the main mechanisms of action. Finally, the main limitations and the needs for future research will be presented.

2. Methods of Obtaining Chitin and Chitosan Used in Agricultural Production as Biostimulants

Chitin and chitosan are produced by two major extraction methods, namely chemical and biotechnological. Chemical processes are based on the use of strong acids and bases are currently the most widely applied methods in both laboratory and industrial scale production [53]. Two well-known methods of chitosan production are to extract chitosan directly from cell walls of molds, and thermo-chemical or enzymatic methods of chitin deacetylation to remove the N-acetyl groups from chitin. At present, chitosan is manufactured industrially through thermo-chemical hydrolysis of chitin's amide bonds [53]. Several forms such as solutions, flakes, fine powder, beads and fibers are available for commercial preparations of chitosan [54]. Chitooligosaccharides can be produced through chemical, physical, electrochemical and enzymatic degradation of chitin and chitosan [53]. The most commonly applied chemical methods of chitooligosaccharides production include acid degradation and oxidation degradation of chitin and chitosan [53].

The traditional systems for commercial preparation of chitosan from various sources may lead to some drawbacks and many disadvantages since they are not cheap or environmentally friendly, and have inconsistent molecular weight and degree of acetylation [55,56]. A promising economical method for innumerable application and the production of highly viscous chitosan is the use of biotechnology fermentation processes, such as deproteinization and demineralization by organic acid bacteria and protease and deacetylation by chitin deacetylase [56,57]. Chitosan can be promoted as a green product [35], and chitosan from crustacean as a food industry waste is economically feasible [58–60]. Although chitosan is mainly obtained from crustacean shells rather than from insect and fungal sources, the commercialization of chitosan extraction from insect and fungal sources has increased in recent years [35]. Techno-economic sensitive approaches have also been performed for chitosan production from shrimp shell wastes [61].

The chemical methods for production of chitin and their derivatives that are currently being applied on a commercial scale consist of two steps, namely, deproteinization by alkali treatment and demineralization by acidic treatment under high temperature, followed by the decolorization step which focuses on removing lipids and pigments [35]. Ambient temperature and stirred bioreactors have been applied to improve the quality and to shorten the process [50,62]. Crustacean wastes from the shrimp and crab industry are pretreated with washing and grinding, and then the grinded exoskeleton goes through depigmentation by ethanol. After that, the exoskeleton proceeds to the demineralization stage by hydrochloric acid, and then the exoskeleton proceeds to the deproteinization stage by sodium hydroxide and provides chitin. Finally, chitin goes through deacetylation by sodium hydroxide and produces chitosan [61,63].

3. Practical Applications of Chitosan on Vegetable Crops

Chitosan is an environmentally and eco-friendly polymer with multipurpose applications in various fields such as agriculture, cosmetology, food, paper, pharmacy and textile industries [64–68] and a potent agent for the removal of toxic pollutants [69,70]. It can be used in plant production systems as a single compound or combined with other polymers and elements [68,71]. It is considered one of the most abundant natural biopolymers. It is derived from chitin and its structure consists of two sub-units, namely D-glucosamine and N-acetyl-D-glucosamine, connected with 1,4-glycosidic bonds to each other [72,73]. Its ability to bind on other compounds allows the delivery of nutrients, pesticides and biomolecules in plants systems [71,74,75]. The precursor of chitosan (chitin) is the second most renewable source of carbon throughout the world, which makes chitosan a very promising material for industrial applications, with more than 2000 tons produced annually [76]. However, the preparation of chitosan via industrial methods produces a final product that cannot be described accurately as chitosan since it contains various

polymers with different degrees of polymerization and physical properties [72,77]. Despite this downside, the benefits from chitosan application are far more important since it is claimed to be GRAS (generally recognized as safe) and easily absorbed, inexpensive, easily available and easy to manipulate [78,79].

The beneficial activities of chitosan are mostly associated with increased photosynthetic activity, tolerance to abiotic stressors such as drought, salinity and extreme temperatures, as well as with increased antioxidant enzymes activity and the expression of defensive genes [80]. There are numerous examples of chitosan application on vegetable crops; however, the obtained results are not always consistent since the various studies differ in their methodological approach (time and dose of application), while chitosan-based biostimulant products may also differ in chemical composition and chitosan content, which further increases heterogeneity in biological effects [77,78,81–83]. The primary use of chitosan in agriculture is based on its eliciting effects on the biosynthesis of protective biomolecules against pests and pathogens [14,84,85], as well as on the up-regulation of defensive genes [86,87].

It can be applied in various forms including seed coating, foliar spraying or soil incorporation and as a coating agent in fruit and vegetables for post-harvest protection [31,88,89]. The main activity of this biopolymer is plant protection against various biotic and abiotic stressors via various mechanisms that must be unraveled. For example, the hydrophilic nature of chitosan may alleviate stress effects by reducing water content in cells [14], while it can also increase root length and reduce the transpiration rate, resulting in improved water uptake and water use efficiency in plants [90,91]. Moreover, chitosan application may result in plant growth improvement mostly through the increased nitrogen and nutrients uptake, while it can be used as an extra carbon source in plant biosynthetic processes [82,92]. Other activities include the effects of mycorrhization in tomato plants through the regulation of the expression of endochitinase-encoding genes [81]. Moreover, the foliar application of chitosan may serve as a physical barrier against pathogens [93], while it can increase the thickness of cell walls in the leaves' epidermis, contributing to tolerance against pathogens attacks [94]. Its use as soil amendment has also found practical applications in agriculture, resulting in increased yield in lettuce [95] and tomato [96] crops, while it can remediate polluted soils through the removal of cationic and anionic heavy metals and the improvement of soil properties [97–99]. Seed coating with chitosan may increase germination percentage and seedling growth through the induction of antioxidant enzymes [100–102], or the increased water absorption through the formation of a semi-permeable coating on the seed surface [103,104]. Finally, coating fresh fruit and vegetables with chitosan may increase shelf life [105], retain the quality and prevent spoilage from food-borne pathogens [106–108] and microbes that affect human health [109].

Apart from the direct effects of chitosan, there are several applications of chitosan derivatives and nanoparticles, which are used as carriers of nutrients and other compounds. Chitosan-based biodegradable nanomaterials (NMs) consist of nanogels, nanospheres, nanocapsules, nanoparticles and nanocomposites, which have been applied for plant growth promotion and plant protection especially against viruses, fungi and bacteria, providing a new and effective tool for sustainable crop protection [7,110,111]. For example, chitosan-coupled copper nanoparticles (ch-CuNPs) have several advantages as a growth promoter and fungicide, showing promising properties for substituting conventional pesticides and ameliorating their hazardous impacts on the environment [112]. Chitosan nanoparticles were also suggested to increase immunity against pathogens through the induction of innate defense mechanisms and defense-related enzymes [97]. Moreover, the application of nanochitosan solutions via soaking of seedlings or foliar spraying showed better results in terms of onion crop performance and nutrient use efficiency [113]. The advanced techniques in nanoparticle preparation have allowed the efficient capitalization of chitosan's beneficial effects not only in agriculture but also in the food industry through functional packaging [67,68,114]. The use of bio-nanomaterials may also find uses in smart genetic engineering in plants through the editing of plant genomes [71]. However, the use

of such materials for human-related purposes is currently restricted and under debate and further studies are needed to transfer the achieved knowledge from the laboratory to an industrial scale and to manifest the positive effects with large-scale trials [71,115,116].

The most notable impacts of chitosan on various vegetable plants are presented in Table 1.

Table 1. The effects of chitosan on vegetable crops.

Plant	Scientific Name	Plant Family	Key Point	Reference
Artichoke	<i>Cynara scolymus</i> L.	Asteraceae	Chitosan application promoted germination and plant growth of artichoke and induced a significant decrease in fungi infections.	[117]
Basil	<i>Ocimum basilicum</i> L.	Lamiaceae	Chitosan may ameliorate harmful impacts of drought on basil growth, as well as improve total phenol and antioxidant activity.	[92]
Bean	<i>Phaseolus vulgaris</i> L.	Fabaceae	Chitosan lactate foliar application may promote the accumulation of bioactive substances; increase the activity of antioxidant enzymes; improve photosynthetic rate and plant growth.	[118]
			Chitosan increased the yield on normal or delayed sowing. Effective impact of nanocarriers composed alginate/chitosan (ALG/CS) in promoting leaf area and the levels of chlorophylls and carotenoids.	[119] [120]
Bell pepper	<i>Capsicum annuum</i> L. var. <i>grossum</i> (L.) Sendt	Solanaceae	Chitosan nanoparticles (CsNPs) indicated the significant role in anti-biofilm activity against foodborne pathogens.	[121]
			Chitosan nano-coating (CsNC) lengthened the shelf life of fresh-cut bell pepper.	[121]
			Chitosan treatments increased germination, improved seedling growth and emergence in cold test.	[122]
Chickpea	<i>Cicer arietinum</i> L.	Fabaceae	CaCl ₂ -tea tree oil (TTO)/low molecular weight chitosan (LMWCS) slowed down the microbial growth in fresh-cut bell pepper.	[114]
			Chitosan nanoparticles-loaded application with thiamine increased germination percentage and growth in chickpea. Foliar application stimulated protection of chickpea seedlings against wilt disease, and increased indole acetic acid (IAA) production in seedlings.	[123] [124]
Chilli	<i>Capsicum frutescens</i> L.	Solanaceae	Chitosan applied as seed treatment (1%) and foliar spray (0.5%) combined application showed the highest effectiveness in controlling anthracnose of chili and stimulated yield and yield contributing characters.	[125]
Cucumber	<i>Cucumis sativus</i> L.	Cucurbitaceae	Chitosan seed treatment resulted in 100% resistance against damping off caused by <i>Phytophthora capsici</i> .	[126]
			Chitosan may synthesize defense-responsive enzymes and stimulate phytohormones in cucumber plants.	[102]
Eggplant	<i>Solanum melongena</i> L.	Solanaceae	The synthesized nanocomposites improved both the nematocidal activity and the plant systematic immune response.	[127]
Faba bean	<i>Vicia faba</i> L.	Fabaceae	The new carboxymethyl chitosan-titanium nanobiocomposites may decrease negative effects of <i>Bean yellow mosaic virus</i> (BYMV).	[128]
Ginger	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Chitosan and oligochitosan suppressed ginger rhizome rot in storage.	[129]
			Chitosan and oligochitosan improved defense enzymes activity in ginger.	[129]
Lettuce	<i>Lactuca sativa</i> L.	Asteraceae	Chitosan application at 2% in a Ni-contaminated soil may significantly regulate Ni bioavailability.	[130]

Table 1. Cont.

Plant	Scientific Name	Plant Family	Key Point	Reference
			Chitosan nanoparticles (CNPs) loaded with indole-3-acetic acid (IAA) indicated a beneficial impact on the hydroponic lettuce growth.	[131]
Onion	<i>Allium cepa</i> L.	Amaryllidaceae	Nano chitosan may improve the efficiency of traditional fertilizers and promoted the net return per fed.	[113]
			Chitosan/polyacrylic acid hydrogel nanoparticles (CS/PAA-HNPs) stimulated the yield, plant growth and nutrient content in onion bulbs.	[132]
Okra	<i>Hibiscus esculentus</i> L.	Malvaceae	Chitosan foliar application at 100 or 125 ppm may be applied at early growth stages to achieve higher yields.	[133]
Pea	<i>Pisum sativum</i> L.	Fabaceae	<i>Ascophyllum nodosum</i> extract (ANE) and chitosan suppressed pea powdery mildew by modulating Jasmonic acid and Salicylic acid-upregulated signaling pathways.	[84]
Pepper	<i>Capsicum annuum</i> L.	Solanaceae	Nano-chitosan positively affected plant morphogenesis, growth and physiology.	[134]
Potato	<i>Solanum tuberosum</i> L.	Solanaceae	Chitosan application may significantly increase root fresh and dry weight.	[135]
			Foliar spraying of chitosan combined with humic acid could lead to higher tuber yield and yield components.	[136]
			Chitosan (75 mg/L) and oligo-chitosan (50 mg/L) can increase plant growth and induce defense mechanisms for drought stress tolerance.	[137]
			Chitosan can inhibit the growth and spore germination and induce resistance against <i>Fusarium oxysporum</i> .	[138]
			Growth and spore germination of <i>Phytophthora infestans</i> were inhibited by chitosan.	[139]
Sweet potato	<i>Ipomoea batatas</i> L.	Convolvulaceae	Chitosan slowed down the cell growth, induced cell necrosis and significantly affected fatty acid composition of <i>Ceratocystis fimbriata</i> .	[140]
Tomato	<i>Solanum lycopersicum</i> L.	Solanaceae	Chitosan had positive effects on plant growth promotion and control of <i>Ralstonia solanacearum</i> .	[141]
			Foliar application of salicylic acid and chitosan at 75 mg L ⁻¹ may be utilized at early growth stage for getting maximum fruit yield in summer tomato.	[90]
			Chitosan ameliorated viral load, stimulated gas exchange and regulated <i>PAL5</i> expression, while it decreased the adverse impacts of <i>Cucumber mosaic virus</i> (CMV).	[142]
			Chitosan indicated the appropriate results to inhibit the infection caused by <i>Rhizopus stolonifer</i> on the tomato fruits.	[143]
			Chitosan combined with chelated copper had a higher efficiency in the enzyme activation associated with pathogenicity than chitosan or copper acting alone.	[94]
			Chitosan + compost + arbuscular mycorrhizal fungi application improved tomato growth.	[144]

4. Activities and Applications of Oligochitosan

Oligochitosan (or chito oligosaccharides), with 3 to 10 saccharide residues of N-acetylglucosamine or glucosamine, include homo or hetero oligomers obtained from chitin by chemical or enzymatic hydrolysis [145], or through oxidative and ultrasonic degradation [146,147]. It is considered a plant elicitor and has similar effects as chitosan on plants against biotic stress and plant growth improvement [148], while it also possesses significant beneficial properties for human health [149]. Moreover, similarly to chitosan, the biological activities of oligochitosan are also dependent on the degree of polymerization (DP) and the acetylation pattern, as well as on the concentration of the applied compound and the plant species [46]. In particular, oligochitosan with higher DP showed stronger elicitation effects through the expression of defensive genes [150].

The main activities of oligochitosan are associated to the induction of secondary metabolites biosynthesis and the activation of plant innate immunity through signal perception and transduction, expression of defensive genes and finally the accumulation of protective secondary metabolites [151,152]. So far, most of the applications refer to field crops and a limited number of studies evaluated the effects of oligochitosan on vegetable crops. For example, oligochitosan application showed higher in vitro effectiveness than chitosan in inhibiting mycelia growth of *Phytophthora* species [153,154], while it promoted plant growth and yield in various vegetable crops such as common bean, potato, tomato, chili pepper, spinach and eggplant [155–158]. The combined application of oligochitosan and ϵ -poly-L-lysine in tomato plants showed synergistic effects against *Botrytis cinerea* infections both under in vitro and in vivo conditions, suggesting their use as a bio-fungicide alternative to synthetic fungicides [159]. Moreover, Li et al. [160] suggested that oligochitosan induced the production of nitric oxide and hydrogen peroxide in *Brassica napus* L. plants, which acted as signaling molecules in the regulation of stomata closure and the expression of *LEA* protein gene for the protection against drought. Apart from protective effects, oligochitosan may improve the functional properties of vegetable products, as already reported in case of white radish sprouts (*Raphanus sativus* L.) where seed germination with oligochitosan-treated water resulted in a significant increase in the most abundant glucosinolate, e.g., glucoraphasatin [161].

The use of oligochitosan has great potential for farming applications, especially in crops with high added value as in the case of vegetable species. However, future research is needed to define important parameters regarding the biostimulant product, such as the degree of polymerization, and fine tune the application practices related to dose and application time and method.

5. The Use of Chitin as Biostimulant

Chitin is a versatile polymer of β -1,4-*N*-acetylglucosamine widely abundant in nature, and mainly obtained from prawn/crab shells for commercial purposes [38,72,162], while the isolation of chitin from edible fungi production chain has also been considered [163,164]. It is composed after the polymerization of *N*-acetylglucosamine through the activity of chitin synthases which are classified in three divisions and seven classes [165]. Chitin is the second most abundant polysaccharide in living organisms after cellulose, being the main structural compound in fungal cells and the skeleton of invertebrates [38,166]. The main differences of chitosan are its hydrophobic nature and the lower solubility in water and several organic solvents, which pose restrictions in practical applications in agriculture and significantly affect the biological properties of chitin [167]. Therefore, its chemical modification and the derivatives obtained through chemical reactions are of major importance towards the better exploitation and valorization of this biopolymer. The current market trends show that the global chitin market is expected to reach USD 2900 million by 2027, with healthcare, waste and water treatment and agrochemicals sectors being the largest market segments [168].

The main application methods of chitin on plants consist of foliar spraying and direct soil application, while it may be also applied on coating horticultural products to increase their shelf life after processing [169–171]. When foliar spraying is applied, the positive effects of chitin are associated with its direct act as a physical barrier against pathogen infections or with indirect activities that induce the plant immune system as signaling molecules for defense pathways [167,172,173]. Soil application effects are more complex than foliar spraying and include (a) the increased bioavailability of nitrogen due to the high content of chitin in this important macronutrient and the low C/N ratio [174], (b) the increased activity of chitinolytic organisms that may have antagonistic effects against plant soil pathogens [175] and (c) the favorable effects on soil microbiota such as mycorrhiza and rhizobia that act synergistically to plant and improve crop performance [176,177]. On the other hand, the use of chitin in edible coatings of horticultural products may provide a semipermeable physical barrier that regulates gas exchange and may delay ripening and

decrease water losses and respiration rates [178]. However, these effects may differ since chitin is a natural product and differences in composition and physicochemical properties (e.g., nitrogen and ash content, degree of deacetylation, bulk density and viscosity) of commercial products may result in differences in biological activities [179].

Several studies have evaluated the biostimulatory effects of chitin on vegetable crops. For example, Rajkumar et al. [85] suggested that combining chitin with salicylic acid may increase the population of *Pseudomonas* sp. strains SE21 and RD41 which act antagonistically against *Rhizoctonia solani*, causing damping off in pepper plants. Moreover, chitin obtained from yeast cell walls may increase the tolerance of tomato fruit against *Botrytis cinerea* [180]. Peat supplementation with chitinolytic plant growth promoting *Bacillus subtilis* AF1 resulted in increased emergence and plant growth of pigeon pea seedlings [181], while the amendment of peat substrate with chitin increased the rhizobiome of lettuce, resulting in improved plant growth [182]. Chitin has also been used in biocontrol agents against soil-borne and foliar plant pathogens and pests [183]. Considering the disadvantageous properties of chitin that limit its direct application in plants, complex structures have been suggested such as the protein/CaCO₃/chitin nanofiber complex which improved plant growth in hydroponically grown tomatoes [184], or polymeric chitin nanofibers which exhibit eliciting activities [185]. Chitin nanofibers were also effective in inhibiting the infections by *Alternaria brassicicola* and *Colletotrichum fructicola* in cabbage and strawberry plants, respectively [186], as well as in increasing the tolerance against *Fusarium* wilt [187] or improving nitrogen use efficiency and promoting the growth of tomato plants [188]. Moreover, the application of a formulation based on chitin and *Trichoderma* ameliorated the occurrence of head rot (*Sclerotinia sclerotiorum* (Lib.) deBary) and root-knot (*Meloidogyne incognita* Kofoid and White; Chitwood), while it also increased the yield of cabbage plants grown under field conditions [189]. The application of betaine and chitin in lettuce plants grown under a regulated water deficit irrigation regime increased crop performance, as expressed by improved water use efficiency values [190]. Chitin oligosaccharide dithicyclobutane derivative showed nematocidal activity against *Meloidogyne incognita* in tomato seedlings, an effect that could be associated with its glutathione binding activity [191].

Table 2 presents the most important effects of chitin application on vegetable crops.

Table 2. The effects of chitin on vegetable crops.

Plant	Scientific Name	Plant Family	Key Point	Reference
Cabbage and strawberry	<i>Brassica oleracea</i> cv. Shoshu and <i>Fragaria</i> × <i>ananassa</i>) var. Yotsuboshi	Brassicaceae and Rosaceae	Chitin nanofibers induced plant resistance against <i>Alternaria brassicicola</i> <i>Colletotrichum fructicola</i> and increased plant growth.	[186]
Cabbage		Brassicaceae	Chitin and <i>Trichoderma</i> formulation reduced the incidence of complex diseases <i>Sclerotinia sclerotiorum</i> and <i>Meloidogyne incognita</i> .	[189]
Chili pepper	<i>Capsicum annum</i> L.	Solanaceae	Chitin and salicylic acid application along with antagonists (fluorescent pseudomonads SE21 and RD41) effectively controlled damping off (<i>Rhizoctonia solani</i>) of seedlings. Soil amendments with chitin effectively controlled <i>Meloidogyne javanica</i> and <i>Fusarium solani</i> infections.	[85] [192]
Eggplant	<i>Solanum melongena</i> L.	Solanaceae	Soil amendments with chitin obtained from crabs suppressed <i>Verticillium</i> wilt in plants.	[193]
Lettuce	<i>Lactuca sativa</i> L.	Asteraceae	Peat supplemented with chitin increased the growth of lettuce plants and siderophore and chitinase genes. Soil application of chitin combined with foliar application of betaine improved crop performance under water stress conditions.	[182] [190]
			The application of chitin-rich residues in growth medium increased lettuce plant growth and improved post-harvest quality.	[194]

Table 2. Cont.

Plant	Scientific Name	Plant Family	Key Point	Reference
Pigeon pea	<i>Cajanus cajan</i> L.	Fabaceae	Peat supplemented with chitin increased seedling emergence and growth of seedlings.	[181]
Tomato	<i>Solanum lycopersicum</i> L.	Solanaceae	Post-harvest treatment of tomato fruit with chitin induced resistance to <i>Botrytis cinerea</i> infections.	[180]
			Foliar application of chitin-based inoculum of <i>Paenibacillus elgii</i> HOA73 inhibited gray mold infections in fruit.	[183]
			Nanofiber complex of protein/CaCO ₃ /chitin increased plant growth through efficient minerals release.	[184]
			Chitin nanofibers induced plant growth through the increased nitrogen use efficiency.	[188]
			Complexes of protein/CaCO ₃ /chitin and protein/chitin nanofiber reduced <i>Fusarium</i> wilt incidence.	[187]
			Combined application of chitin and chitosan reduced the incidence of <i>Rhizoctonia solani</i> , <i>Fusarium solani</i> and <i>Sclerotium rolfsii</i> in plants.	[195,196]
			Chitin incorporation in the soil reduced root galls from nematode infections.	[197]

Figure 1 shows the most notable advantages of chitin and chitin derivatives application, while the chemical structures of chitin (C₈H₁₅NO₆) and chitosan (C₅₆H₁₀₃N₉O₃₀) are shown in Figure 2.

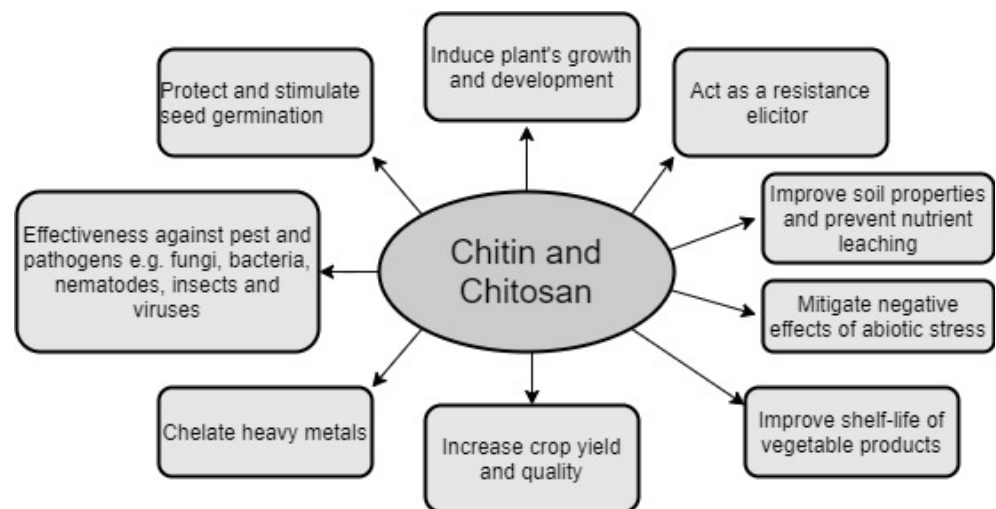


Figure 1. The most important effects of chitin and its derivatives' applications.

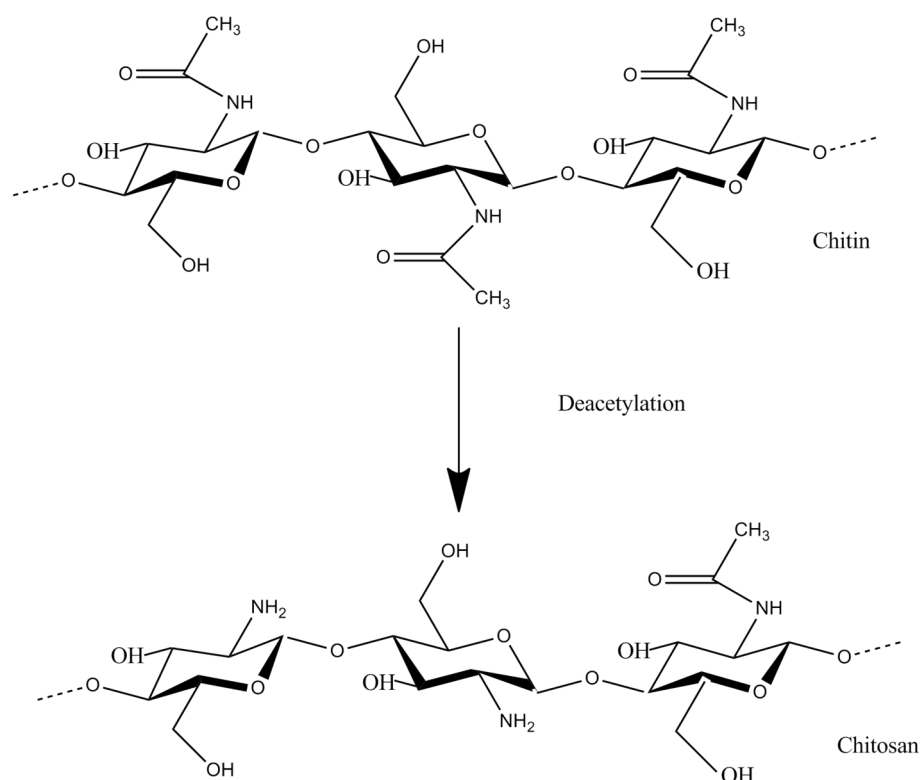


Figure 2. The chemical structure of chitin ($C_8H_{15}NO_6$) and chitosan ($C_5H_{10}N_2O_3$).

6. Conclusions

Chitin, chitosan and chitosan oligosaccharides are natural biopolymers with numerous activities in plants. So far, the practical applications of these compounds have shown beneficial effects on the protection of horticultural plants against pathogens, and on plant productivity and growth, especially under environmental constraints which highlight its promising roles for crop cultivation under drought conditions in arid and semi-arid regions. Among these compounds, chitosan seems to be the most economical option for improving productivity and quality of various plants at the moment, especially in high added value species such as horticultural crops. On the other hand, chitin single applications are limited mostly due to practical limitations related to the hydrophobic and insoluble nature of this compound. Therefore, various transformations must be considered in order to valorize its beneficial effects on plants through the complexation with other compounds or the nanofibrillation. Moreover, the diverse sources of chitin and derivatives make it difficult to standardize the composition of commercial products, which may affect their biological activities in plants. Finally, the farming sector is a very promising alternative to exploit these natural biopolymers and provide farmers a sustainable tool to increase crop productivity and the quality of the final product. However, further studies are needed to improve reproducibility of the positive effects and to standardize the production processes from the lab to an industrial scale. Both of these aspects will help towards improving the application protocols of biostimulant products with standardized composition.

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