

Chitin- and Chitosan-Based Derivatives

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This review deals with the utilization of chitin and chitosan derivatives in agriculture. In particular it summarizes recent results concerning the utilization of these compounds to face biotic and abiotic stresses. In addition the review summarizes recent results about the utilization of chitin- and chitosan-based materials for the recovery of waste water and soil contaminated by different chemicals

Keywords: chitin ; chitosan ; defense responses ; plant growth ; recovery ; stress

1. Introduction

Biotic (pathogen attack, herbivores, wounding), abiotic (deficient or excessive water, low or high temperature, high salinity, ultraviolet radiation, heavy metals, various toxic contaminants) stresses and in particular the unpredictable combination of different stresses are very harmful to plant growth and development, and lead to severe crop yield loss worldwide^[1]. In consideration of the increasing food demand of the growing world population, it is becoming imperative to enhance crops' tolerance of multistress. So far, different approaches have been tested to increase plant resistance against stresses. In particular, an increasing resistance has been obtained by the use of different chemicals such as pesticides, fertilizers and phytochemicals. However, the extensive use of these chemicals in agriculture has a great environmental impact, with accumulation in soil, water and in living organisms^[2]. This has stimulated the search of more eco-friendly mechanisms to manage plant stresses. Chitin, a naturally occurring long-chain high molecular weight polysaccharide composed of *N*-acetyl-D-glucosamine and D-glucosamine, a main component of the exoskeleton of arthropods and of the fungal cell wall, and its deacetylated derivative chitosan, seem promising tools to solve this problem (Figure 1).

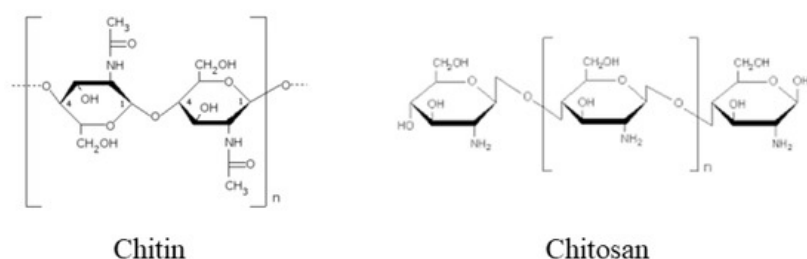


Figure 1. Chemical structure of chitin and chitosan.

In fact, these molecules, easily obtainable from the crustacean shells of crabs, prawns etc., and are non-toxic, biodegradable and biocompatible. This accounts for their potentially broad application in agriculture where they can act as potent stimulators of plant productivity and protectors against pathogens (^{[3][4]}and references therein). In addition, chitin and chitosan can act as bioadsorbents for remediation of contaminated soil and water. The increasing industrial production and use of synthetic molecules such as dyes, pesticides, fertilizers, molecules containing heavy metal ions or nuclear residues are a major environmental concern. In fact, these compounds accumulate in soil and water and enter into food chains resulting in mutagenesis, carcinogenicity and other serious human health impairments^[5]. To date, arrays of methodologies are in use to remediate this situation. Adsorption is the most attractive, in particular when it employs eco-friendly, sustainable, and low-cost materials such as chitin and chitosan^[6].

After some pioneering indications in the early 1980's on the protective effects of chitin and chitosan, an impressive number of papers dealing with the use of these molecules in an agricultural context have been published. These papers have been summarized in many exhaustive reviews (^{[2][3][4]}, among others), thus in this review we summarize only recent results (years 2018–2020) obtained using chitin- and chitosan-based derivatives in plant protection against biotic and abiotic stresses and in recovery of contaminated soil and water.

2. Chitin- and Chitosan-Based Derivatives in Plant Protection against Biotic Stress

The total world population has increased from 2.5 billion in the 1950's to over 7 billion in the present and is expected to increase to 9 billion by the end of the century. To feed this increasing population, food production must also rise, and in a safe and environmentally sustainable manner. At present, severe crop yield losses occur due to plant diseases and to global climate changes that have increased some phytosanitary emergencies^[7]. To fight these losses, several chemicals are still in use in modern agriculture. However, these agrochemicals are not without risk for the environment where their residues can easily accumulate. Thus, the implementation of novel strategies to manage plant disease is crucial to respond to the growing demand of safe and healthy food. In this perspective, chitin and chitosan are among the most promising tools. In fact, they can fight several stresses permitting relevant increases in plant productivity. The exact action mechanism of these protective molecules is under investigation and the different possibilities summarized in the following have been proposed (see^[4] and references therein). Chitin and chitosan are positively charged molecules that can easily interact with the anionic structures present in the cell wall and in the cellular and nuclear membranes of pathogens like proteins, lipopolysaccharides and negatively charged ions. This can lead to leakage of intracellular components and the death of the microorganism. Interestingly, the negatively charged phosphate groups of nucleic acids can strongly interact with chitin and chitosan. This direct interaction can induce specific modifications in the expression and activity of proteins involved in the stress response. In addition, recent investigations strongly suggest the presence in the cellular plasma membrane of a specific receptor belonging to the glycoprotein family of lectins. The binding with this receptor starts a well-defined signaling cascade that leads to the responses. Finally, it should be noted that chitin and chitosan with their high nitrogen content and very low C/N ratio can directly act as natural fertilizers (^[8] and references therein). Whatever the mode of action, chitin, chitosan and their derivatives (e.g., nanoparticles ($\leq 0.5 \mu\text{m}$ in size), microparticles ($\geq 1 \mu\text{m}$ in size) and oligosaccharides, degradation products formed by no more than 12 glucosamine residues) permit a relevant increase in plant productivity by controlling several plant pathogens ([Table 1](#)).

Table 1. Chitin- and chitosan-based derivatives in plant protection against biotic stress.

Plant Species	Characteristics of the Protective Molecules and Method of Administration	Protective Effect	Reference
<i>Capsicum annuum</i> L.	1% chitosan, foliar application	Resistance against <i>Phytophthora capsici</i>	[9]
<i>Melissa officinalis</i>	0.005, 0.01, 0.015% chitosan, shoot spraying	Accumulation of defense-related enzymes and phenolic compounds	[10]
<i>Phoenix dactylifera</i> L.	0.1% chitosan nanoparticles, seedling irrigation	Enhancement of the innate immunity	[11]
<i>Solanum lycopersicum</i>	0.001, 0.01, 0.1% chitosan microparticles, foliar application	Accumulation of defense-related enzymes	[12]
Stone fruit trees	0.001% chitosan-Ag nanoparticles, foliar application	Resistance against <i>Pseudomonas syringae</i>	[13]
<i>Beta vulgaris</i>	0.2% chitosan; 0.05% nano chitosan, foliar spraying	Resistance against <i>Pegomya hyoscyami</i>	[14]
<i>Solanum tuberosum</i> L.	0.4% chitosan, tuber immersion	Resistance against <i>Fusarium</i> spp.	[15]
<i>Oryza sativa</i> L.	0.3% chitosan oligosaccharide, seedlings spraying	Resistance against <i>Fusarium oxysporum</i>	[16]
<i>Citrus reticulata</i> Blanco	0.05% chitin oligosaccharide, leaf infiltration	Resistance against <i>Candidatus Liberibacter asiaticus</i>	[17]
"in vitro" test	0.5% chitin oligosaccharide diluted in culture medium	Inhibition of <i>Botrytis cinerea</i> spores germination	[18]

Foliar application of chitosan enhances growth and modulates expression of defense genes in chilli pepper (*Capsicum annuum* L.) thus reducing the severe losses in chilli production induced by *Phytophthora capsici* infection^[9]. Shoot cultures of *Melissa officinalis* treated with chitosan accumulate several defense-related enzymes and phenolic compounds with antimicrobial activity^[10]. Similar results have been obtained in date palm (*Phoenix dactylifera* L.) seedlings treated with chitosan nanoparticles^[11] and in tomato (*Solanum lycopersicum*) seedlings treated with chitosan microparticles^[12]. A silver-chitosan nanocomposite reduces canker disease induced by *Pseudomonas syringae* pv. *syringae* in stone fruit trees^[13] and chitosan and nano chitosan treatments efficiently control beet fly (*Pegomya hyoscyami*) infection in

sugarbeet (*Beta vulgaris*) plants^[14]. Similarly, chitosan alleviates diseases induced by *Fusarium* spp. in potato (*Solanum tuberosum* L.)^[15] and by *Fusarium oxysporum* in rice (*Oryza sativa* L.)^[16]. Interestingly, in Sun Chu Sha mandarin (*Citrus reticulata* Blanco) plants, hexaacetyl-chitohexaose, a chitin-derived oligosaccharide, affects the vitality of Asian citrus psyllid (*Diaphorina citri*), the hemipteran vector of *Candidatus Liberibacter asiaticus*, the pathogen associated with citrus greening disease^[17]. In addition, efficacy of undiluted chitin-based cultures of *Paenibacillus elgii* HOA73 bacteria, a biocontrol agent that limits the damage caused to plants by microbial pathogens, insects, and nematodes, is comparable to that of standard chemical pesticides, suggesting a possible alternative to these chemicals in eco-friendly agriculture^[18]. The suppressive effect of chitin added to the soil against pathogens often involves a change in the composition of the soil microbiota with an increase in the presence and activity of chitinolytic microorganisms that hydrolyze the chitinous hyphae of pathogenic fungi, and with an increase in secondary responders to added chitin that may affect pathogens^[19]. However, it has been reported that deacetylation by specific enzymes of chitin oligomers converting them to ligand-inactive chitosan, is a strategy largely used by soil-borne fungal pathogens to prevent the protective effect of chitin^[20]. These results indicate that more investigations are needed to exactly clarify the virulence strategy of soil-borne fungal pathogens.

3. Chitin- and Chitosan-Based Derivatives in Plant Protection against Abiotic Stress

To secure healthy and safe food of high nutritional quality to the growing world population and in particular, to consent adequate food access even in the present condition of global climate changes, it is necessary to increase plant resistance against abiotic stress too. In fact, abiotic stresses (temperature, water, salt, heavy metals, and UV radiation, among others) account for relevant losses in agricultural production worldwide^[1]. To this end, in addition to selection of more performing crop genotypes, evaluation of new agronomic techniques and/or new agrochemicals without adverse ecological impact is needed. Water scarcity is regarded as the key restriction point for food production worldwide^[21]. Thus, many investigations have been recently performed to ameliorate water utilization. Among the investigated substances, chitin and chitosan are able to confer tolerance to several abiotic stresses ([Table 2](#)).

Table 2. Chitin- and chitosan-based derivatives in plant protection against abiotic stress.

Plant Species	Characteristics of the Protective Molecules and Methods of Administration	Protective Effect	Reference
<i>Zea mays</i> L. cv. White Pearl	0, 2 and 4 g chitin added to 1 kg of soil	Drought stress tolerance	[22]
<i>Triticum aestivum</i> L.	0.0125% chitosan, foliar application	Drought stress tolerance	[23]
<i>Triticum aestivum</i> cv. pishtaz	0.0009% chitosan nanoparticles, soil and foliar application	Drought stress tolerance	[24]
<i>Zea mays</i> L.	0.01% chitosan, foliar application	Drought stress tolerance	[25]
<i>Sesamum indicum</i> L.	0.00048, 0.00064% chitosan, foliar application	Drought stress tolerance	[26]
<i>Origanum majorana</i>	0.005, 0.02, 0.05% chitosan, plant irrigation	Drought stress tolerance	[27]
<i>Brassica napus</i> L.	0.2% chitosan, seedling soaking	Drought stress tolerance	[28]
<i>Arabidopsis thaliana</i>	0.01% chitin, plant spraying	Drought stress tolerance	[29]
<i>Triticum aestivum</i> L., <i>Zea mays</i> L.	25, 50, 75% chitosan, seed coating	Salt stress tolerance	[30]
<i>Zea mays</i> cv. Arifiye	0.1% chitosan, foliar application	Salt stress tolerance	[31]
<i>Solanum lycopersicum</i> Mill.	Chitosan–aggregated growth-promoting bacteria	Salt stress tolerance	[32]
<i>Zea mays</i> L.	0.01% chitosan, seedling soaking	Cadmium stress tolerance	[33]

Plant Species	Characteristics of the Protective Molecules and Methods of Administration	Protective Effect	Reference
<i>Solanum melongena</i> L.	0.0125, 0.0150, 0.02% chitosan, foliar application	Heat stress tolerance	[34]
<i>Capsicum annuum</i> L.	0.00125, 0.00250, 0.00375% chitosan, plant spraying	Heat stress tolerance	[35]
<i>Solanum lycopersicum</i> Mill.	0.003, 0.006, 0.009, 0.012% chitosan, foliar application	Heat stress tolerance	[36]
<i>Solanum tuberosum</i> L.	0.25, 0.5% chitosan, foliar application	Poor soil tolerance	[37]
<i>Phaseolus vulgaris</i> cv. Contender	10% chitosan nanoparticles loaded with NPK fertilizers, seed priming and foliar application	Poor soil tolerance	[38]
Mokara Orchids Hybrids	0.002, 0.004, 0.008% chitosan, foliar application	Poor fertilization tolerance	[39]
<i>Solanum lycopersicum</i>	1 mg/plant of chitosan, applied to the soil in the transplant cavity	Poor fertilization tolerance	[40]
<i>Fragaria x ananassa</i> Duch. cv. Elsanta	0.001% chitosan, foliar application	Poor fertilization tolerance	[41]

For example, chitin added to the soil at the beginning of the experiment induces water-stress tolerance in maize (*Zea mays* L.) plants grown under regulated deficit irrigation^[22]. Chitosan application has the potential to mitigate the water deficit effects on yield and quality of wheat (*Triticum aestivum* L.)^[23], and in the same plant species chitosan nanoparticles decrease the adverse effects of drought stress^[24]. Similarly, leaf application of chitosan renders maize (*Zea mays* L.) hybrids more tolerant to water stress^[25] and decreases the plant damage under drought stress in sesame (*Sesamum indicum* L.)^[26]. Chitosan furnished as water solution alleviates water stress in marjoram (*Origanum majorana* L.)^[27], and application of chitosan solution during sowing increases resistance against drought stress and the amount of oil in rape (*Brassica napus* L.)^[28]. Basic studies conducted in the model plant *Arabidopsis thaliana* suggest that chitin modulates water balance through its action on vascular bundle sheath and mesophyll cells^[29]. About other stresses, chitosan application alleviates salt stress thus improving growth performance in *Triticum aestivum* L. and *Zea mays* L.^[30], and confers tolerance to salt stress in maize seedlings by enhancing the expression and activation of alternative oxidase^[31]. Interestingly, inoculation of chitosan-immobilized plant growth-promoting bacteria improves growth of tomato plant (*Solanum lycopersicum* Mill.) under salt stress conditions suggesting the use of this eco-friendly and sustainable approach to fight salt stress^[32]. Finally, chitin and chitosan use permits the cultivation of crops even in non-optimal conditions and the conservation of energy and other resources. For example, chitosan treatment alleviates cadmium stress in maize (*Zea mays* L.) seedlings, permitting their growth in areas subject to heavy metal stress^[33] and application of chitosan protects eggplants in field (*Solanum melongena* L.) against heat and high irradiance stresses^[34]. Chitosan spraying on sweet pepper plants (*Capsicum annuum* L.) permits their growth in unheated greenhouse conditions^[35]. Similarly, foliar application of chitosan or chitosan nanoparticles has positive effects on different plant species. In fact, it ameliorates growth and the quality of tomato (*Solanum lycopersicum* Mill.) plants under plastic tunnel conditions^[36]. It also stimulates tuber yields of potato (*Solanum tuberosum* L.) plants grown under newly reclaimed sandy soil conditions^[37]. Finally, when supplemented as nanoparticles loaded with an NPK fertilizer, it ameliorates growth and productivity of French bean (*Phaseolus vulgaris* cv. Contender) plants grown in clay–sandy soil^[38]. Last but not least, chitin and chitosan treatments permit relevant savings in the use of very expensive and high ecological impacting chemical fertilizers and cultivation in soil with limited nutrients. Chitosan ameliorates inflorescence quality and commercial half-life of Mokara orchid hybrids grown at half the regular application fertilizer dosage^[39]. Chitosan stimulates growth of tomato (*Solanum lycopersicum*) plants^[40] in poor soil conditions, and it promotes growth, fruit yield and quality in strawberry plants (*Fragaria x ananassa* Duch.) cv. Elsanta grown under nutrient limitations^[41].

Chitin and chitosan can protect plants against abiotic stress with different mechanism with respect to different stresses. For example, their foliar application induces drought tolerance by direct antitranspirant coating, induction of stomatal closure, accumulation of stress protective enzymes and metabolites (^[42]and references therein). Similarly, chitin and chitosan can affect heat stress by abscissic acid accumulation, which is linked with the previous reported induction of stomatal closure (^[42] and references therein). Salinity stress is relieved by accumulation of antioxidant enzymes and reduction of lipid peroxidation (^[42] and references therein). Finally, due to the presence of functional amino and hydroxyl groups, chitin and chitosan are also able to form complexes with several heavy metals, thus reducing their bioavailability and alleviating their phytotoxicity (^[42]and references therein).

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