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Chitosan Is the Ideal Resource for Plant Disease Management under Sustainable Agriculture

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Abstract

In the spirit of returning to nature and using scientific applications to raise plant efficiency and reduce pathogen risk, scientists began searching for safe, natural alternatives to pesticides that are highly effective and low cost. On top of these alternatives, chitosan came with its biodegradability, biocompatibility, antimicrobial activity, and nontoxicity, which granted it dual energetic effects during the host-pathogen interaction. Chitosan promotes plant growth, regulates plant cell homeostasis and metabolic processes, and triggers plant defense mechanisms; on the other hand, it inhibits the ability of pathogens by disrupting pathogen growth and reducing reproduction, wherefore chitosan will become an increasingly prevalent and ideal resource for agricultural sustainability.

Keywords: chitosan, eco-friendly, antimicrobial activity, abiotic stress, defense responses

1. Introduction

Diverse plant species are adversely affected by phytopathogens on a large scale, which results in severe economic loss and microbial toxins in food and animal feed. The conventional approaches to plant disease control are no longer efficient and secure, especially in light of the occurrence of severe climatic changes. Researchers had to change their traditional thinking in the process of combating plant diseases, which depends mainly on the use of chemical pesticides. In line with the strategy of sustainable agriculture, natural compounds, especially chitosan, have been put into use as safe alternatives that are effective in control, improving plant properties, and keeping the biological balance between plants and beneficial microorganisms in the soil.

Chitosan is a natural, biodegradable, and environmentally eco-friendly polysaccharide obtained from the exoskeleton of crustaceans such as shrimp, shellfish, crabs, cuttlefish, squid pen, and crawfish through the deacetylation process of chitin [1]. Moreover, chitosan can be produced from fungal chitin [2]. Chitosan has a unique nature as it is a linear polymer consisting of two subunits linked together, namely D-glucosamine and N-acetyl-D-glucosamine [3]. Chitosan concentration, chemical modification, acetylation degree, and molecular weight have all been

identified as critical factors in the suppression of plant pathogen infection in the host plant [4, 5]. In sustainable agriculture, chitosan can be used alone or in combination with other compounds in plant nutrition with the aim of withstanding abiotic stress, stimulating plant defense systems, combating plant diseases, promoting plant seed germination and seedling growth [6, 7], and increasing crop production and quality. In addition, chitosan can purify soil and agricultural wastewater from heavy metals such as mercury, copper, uranium, and lead and thus can be reused for irrigation [8].

2. Chitosan chemical, physical, and biological features

Chitosan has many properties that have captured the interest of researchers over the past 20 years to explore the prospect of using it in a variety of scientific and practical sectors. Chitosan properties depend on several very important factors, such as molecular weight, degree of deacetylation, and solubility. The molecular weight of chitosan might influence the crystal size and morphological character of chitosan-based thin-film composites (TFCs) and other products or membranes [9]. The chitosan molecular weight may range between 50 and 2000 kDa depending on the source of chitin, and it has numerous influences on the viscoelastic properties of solutions and hydrated colloidal forms [10]. The degree of deacetylation determines the content of free $-NH_2$ groups in the polysaccharide and it has an influence on all the functional properties of chitosan [9]. Chitosan can dissolve in aqueous solutions, but it does so more readily in acidic media than in neutral or basic media [11]. The solubility of chitosan varies according to several factors, including polymer molecular weight, degree of acetylation, pH, temperature, and polymer crystallinity. Chitosan has unique chemical properties, e.g., linear polyamine, reactive amino and hydroxyl groups, and chelated metal ions. In addition, the biological properties of chitosan are biocompatibility, biodegradability, antimicrobial activity, biosafety, and nontoxicity [12]. These biological properties vary in influence on plants and their fungal and fungal-like microorganisms, bacterial, viral, viroid, and nematode pathogens according to the physical and chemical features of chitosan. Thus, chitosan compounds are strongly recommended to be used in the management strategies against phytopathogens such as viruses [4], bacteria [13], and fungi [14].

3. Antimicrobial activity of chitosan against phytopathogens

Several hypotheses have been postulated to explain the mechanism by which chitosan affects several phytopathogens:

- a. Chitosan has polycationic nature enables it to interfere with electronegative charges on the outer surface of the microbial cell. The external electrostatic interaction between the positive amino glucosamine groups $-NH_3^+$ of chitosan and the negative charge on the cell surface exists in teichoic acids in gram-positive bacteria, lipopolysaccharides in gram-negative bacteria, and phospholipids in the fungal cell membrane (**Figures 1 and 2**), leading to changes in cell permeability and leakage of intracellular electrolytes and proteinaceous constituents and cell death [15, 16]. Divya et al. [17] suggest that the antimicrobial properties of chitosan are due to the repeated amino groups on the backbone of the polymer structure.

b. Chitosan functions as a chelating agent of metals and vital nutrients, causing microbial starvation and impairing microbial development [18] as the amine

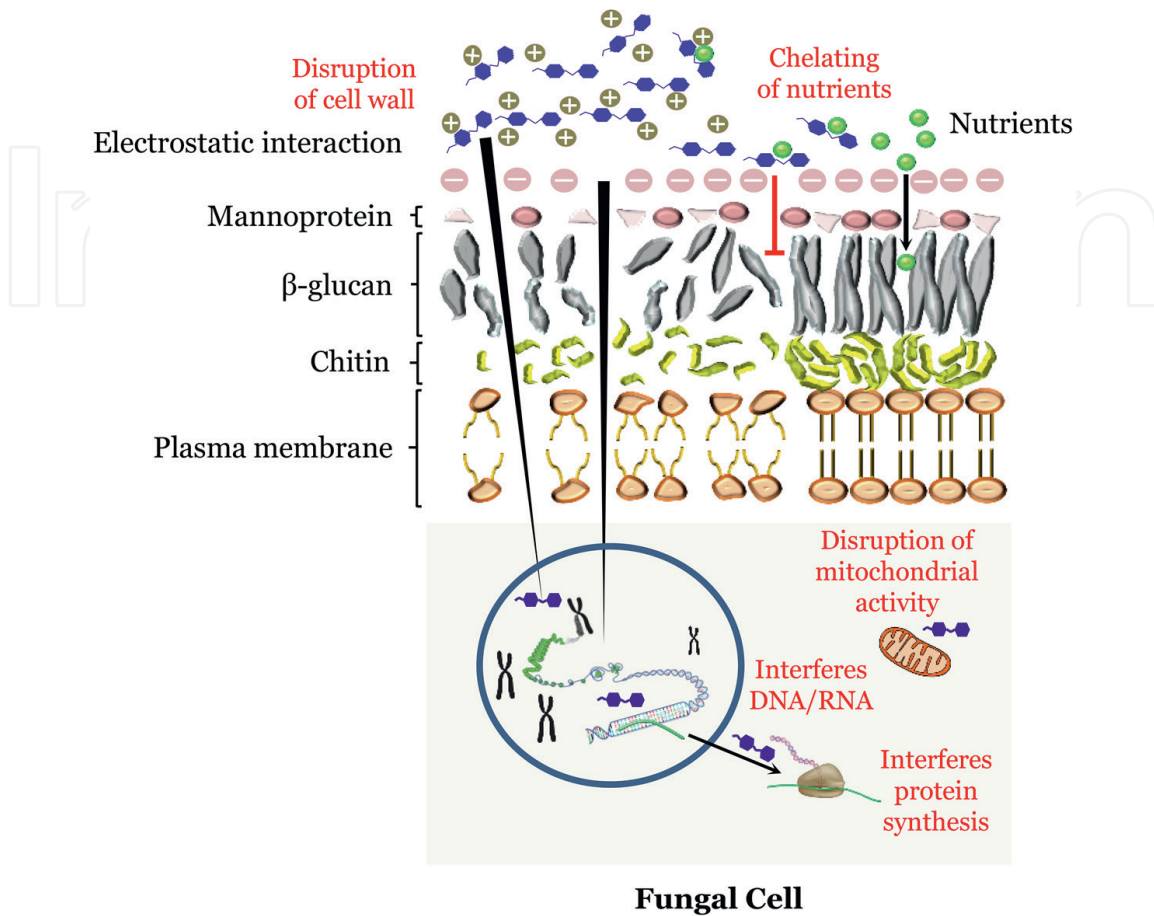


Figure 1.
 Potential antifungal activity of chitosan.

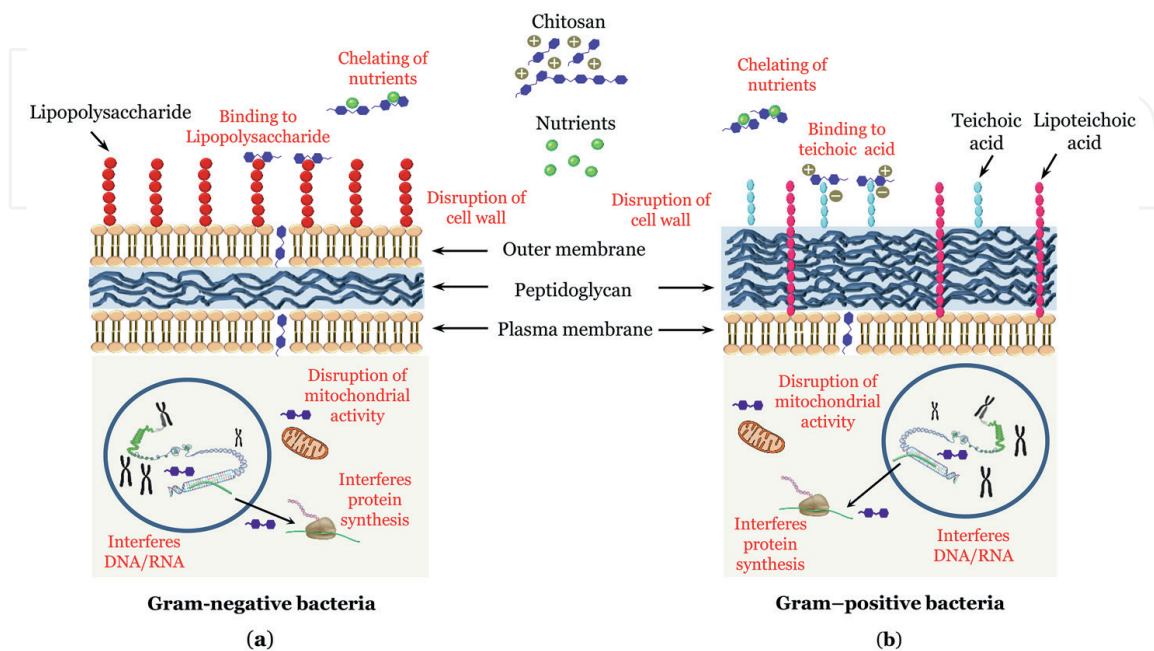


Figure 2.
 Antibacterial effects of chitosan against (a) gram-negative bacteria and (b) gram-positive bacteria.

groups in the chitosan molecules are in charge of the uptake of metal cations by chelation. Chitosan metal-binding capacity increases at high pH since the amine groups are not protonated and the electron pair on nitrogen in the amine group is available for donation to metal ions [19]. The high molecular weight of chitosan might cause a reduction in cell membrane permeability due to a polymer coating on the surface of the cells that blocks cell access to nutrients [20].

- c. The internal electrostatic interactions between the positive amino groups on the polysaccharide chain of low molecular weight chitosan and the negative phosphate groups on the nucleic acid chain of microbial cells lead to the inhibition of the synthesis of DNA/mRNA and a decrease in the abundance level of protein and enzymes [21].

Not all forms of pathogens are equally sensitive to chitosan (**Table 1**); therefore, the degree to which pathogens react to chitosan depends on a number of factors:

- a. The solubility of chitosan increases in acidic solutions and becomes insoluble in solutions with a pH greater than 6.5, according to the pKa values of its amino groups, which range from 6.3 to 6.5. Many articles have shown that chitosan is a great antimicrobial agent under acidic conditions [21]. Meanwhile, the pathogen inhibitory effect of chitosan depends on the type of solvent. Chitosan dissolved in lactic acid shows the best inhibitory effect as compared to that dissolved in formic acid and acetic acid [22].
- b. The ability of chitosan to exhibit intracellular antimicrobial action depends on its molecular weight. Low molecular weight chitosan has the highest inhibitory effect on *Rhizopus stolonifera* [23], while high molecular weight chitosan shows better efficacy on *Fusarium oxysporum* f. sp. *vasinfectum*, and *Alternaria solani* [25].

Furthermore, the abundance of polysaccharides and proteins that make up the numerous layers of the cell wall in both fungi and bacteria has a major impact on the mechanical strength of interaction with chitosan [73].

- c. The degree of deacetylation affects the antimicrobial properties of chitosan. Chitosan with a high degree of deacetylation exhibits a more positive charge, which results in stronger electrostatic interactions with the microbial cell surface and higher antimicrobial activity [13].
- d. The inhibition rate of pathogen growth depends on the concentration of chitosan [74].

3.1 Antifungal activity

Chitosan is efficient in inhibiting hyphal growth, mycelial elongation, spore formation, spore germination, spore viability, germinal tube, and fungal virulence factor production of phytopathogenic fungi [14]. The ability of chitosan to penetrate the plasma membranes of phytopathogens depends on the degree of membrane fluidity. Chitosan-sensitive fungi possess polyunsaturated fatty acid-rich membranes such as linoleic acid (high fluidity membrane), while chitosan-resistant fungi possess saturated

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Tomato wilt	<i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Antifungal	Chitosan was bound to negatively charged phospholipids that alter plasma membrane fluidity and induced membrane permeabilization.	[15]
Potato dry rot	<i>Fusarium sulphureum</i>	Antifungal	Chitosan caused morphological changes such as intertwisting hyphal, distortion, and swelling with excessive branching, abnormal distribution of cytoplasm.	[22]
Post-harvest fungi of fruits and vegetables	<i>Rhizopus stolonifer</i>	Antifungal	Low molecular weight chitosan caused inhibition of mycelial growth, while the high molecular weight chitosan affected spore shape, sporulation, and germination.	[23, 24]
Early blight of potato, tomato, capsicum, and eggplant	<i>Alternaria solani</i>	Antifungal	Chitosan reduced hyphal growth, inhibited sporulation and spore germination, and induced morphological changes.	[25, 26]
Cotton wilt	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	Antifungal		
Apple dieback	<i>Valsa mali</i>	Antifungal		
Banana wilt	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	Antifungal	Chitosan formed abnormal shapes, vesicles, and empty cells devoid of cytoplasm in the mycelia and agglomeration of hyphae.	[27]
Soybean sudden death syndrome	<i>Fusarium solani</i> f. sp. <i>glycines</i>	Antifungal	Chitosan was able to induce the level of chitinase activity in soybean. Furthermore, chitosan interfered directly with the fungal membrane function and interacted with fungal DNA and mRNA.	[28]
Fruit rots in mango, strawberry, apple, and peach	<i>Alternaria alternata</i>	Antifungal	Chitosan caused aggregation of mycelium and structural changes such as excessive branching, cell wall swelling, and hyphae size reduction.	[24, 29]
	<i>Botrytis cinerea</i>	Antifungal		[24]
	<i>Penicillium expansum</i>	Antifungal		[24]
Bayoud disease in date palm	<i>Fusarium oxysporum</i> f. sp. <i>albedinis</i>	Antifungal	Chitosan elicited a defense reaction against this fungus in date palm roots, by eliciting peroxidase and polyphenoloxidase activity and increasing the level of phenolic compounds.	[30]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Cucumber wilt	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Antifungal	Chitosan caused hindering of the <i>Fusarium</i> cell wall, disrupting DNA, disrupting structural and functional protein biosynthesis, and influencing metabolic pathways.	[31]
Early blight disease	<i>Alternaria solani</i>	Antifungal	Chitosan covered the cell wall, causing membrane disruption and cell leakage. Moreover, chitosan penetrated into fungal living cells, leading to the inhibition of various enzymes and interference with the synthesis of mRNA and proteins.	[29]
Root rot disease	<i>Fusarium oxysporum</i>	Antifungal		
Damping off disease	<i>Pythium debaryanum</i>	Antifungal		
Postharvest decay of oranges	<i>Penicillium citrinum</i> , <i>Penicillium mallochii</i>	Antifungal	Chitosan inhibited the growth of two <i>Penicillium</i> species by compacting fungal spores and reducing the activity of plant cell walls by degrading enzymes produced by fungi. Additionally, chitosan increased the wound healing process in orange fruits.	[32]
Anthraxnose and ripen fruit rot in chilli	<i>Colletotrichum capsici</i>	Antifungal	Chitosan completely inhibited the mycelial growth of <i>C. capsici</i> by forming physical barriers around the penetration sites of the pathogen, preventing them from spreading to healthy tissues. In addition, chitosan activated the defense-related antimicrobial compounds, induced a decline in malondialdehyde content, and increased the concentrations of soluble sugars and proline, as well as peroxidase and catalase activities.	[33]
Anthraxnose of camellia	<i>Colletotrichum camelliae</i>	Antifungal	Chitosan enhanced the activity of H ₂ O ₂ , the defense-related enzymes, such as polyphenol oxidase, peroxidase, catalase, phenylalanine ammonia-lyase, and the concentration of soluble proteins inside the camellia plant.	[34]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Fasciation of <i>Arabidopsis thaliana</i>	<i>Staphylococcus simulans</i>	Antibacterial	Chitosan caused multiple changes in the expression profiles of <i>Staphylococcus aureus</i> SG511 genes involved in the regulation of stress and autolysis, as well as genes associated with energy metabolism were observed.	[35]
Common scab in potato tubers	<i>Streptomyces scabies</i>	Antibacterial	Chitosan reduced disease incidence and disease severity by eliciting plant defense mechanisms and systemic resistance.	[36]
Bacterial leaf blight and leaf streak in rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> , <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>	Antibacterial	Chitosan caused membrane lysis and the destruction of biofilm of bacteria and increased the activities of phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase in rice seedlings.	[37]
Bacterial leaf spot of tomato	<i>Xanthomonas vesicatoria</i>	Antibacterial	Chitosan induced systemic resistance mechanisms in tomato by activation of several defense enzymes and elicited expression of PIN II and ETR-1 genes from several molecular pathways involved in pathogen defense.	[26]
Bacterial head rot of broccoli	<i>Pseudomonas fluorescens</i>	Antibacterial	Chitosan reduced the disease incidence, the lesion diameter and induced systemic resistance mechanisms in broccoli.	[38]
Bacterial brown stripe of rice	<i>Acidovorax avenae</i> subsp. <i>avenae</i>	Antibacterial	Chitosan caused membrane disruption and lysis, reduction of biofilm formation, and gene expression change.	[39]
Bacterial fruit blotch of watermelon	<i>Acidovorax citrulli</i>	Antibacterial	Chitosan significantly inhibited the growth of <i>A. citrulli</i> by damaging and altering the cell membrane, separating the cytoplasmic membrane from the cell envelope, coagulating the cytosolic components, forming a vacuole-like structure, and broking of cell walls, leading to the leaching out of a mass of nutrient and nucleic materials.	[40]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Bacteria of crown gall disease	<i>Agrobacterium tumefaciens</i>	Antibacterial	Chitosan was bound with negatively charged components on the bacterial surface, via electrostatic interactions, causing changes in the permeability of the bacterial wall and shutting down the cell division, leading to bacterial fatality.	[29]
Soft mold disease	<i>Erwinia carotovora</i>	Antibacterial		
Bean mild mosaic virus	BMMV	Antiviral	Chitosan led to the inhibition of systemic propagation of viruses over bean plants and enhanced the hypersensitivity response of bean plants to viral infection.	[5]
Tobacco mosaic virus in <i>Arabidopsis thaliana</i>	TMV	Antiviral	Chitosan induced TMV resistance in wild-type and jasmonic acid pathway-deficient (<i>jar1</i>) <i>Arabidopsis</i> plants enhanced the expression of the defence-related gene PR1.	[41]
Alfalfa Mosaic Virus in <i>Nicotiana glutinosa</i>	AMV	Antiviral	Chitosan induced systemic acquired resistance, increased total carbohydrates and total phenolic contents, as well as triggered the transcriptional levels of peroxidase, pathogen-related protein-1, and phenylalanine ammonia-lyase.	[42]
Banana root-knot nematode	<i>Meloidogyne incognita</i>	Antinematode	Chitosan suppressed root-knot nematodes by decreasing the total number of galls, immature stages, females with egg masses, and the count and rate of build-up of nematodes in the soil.	[43]
Tomato root-knot nematode	<i>Meloidogyne javanica</i>	Antinematode	Chitosan reduced the number of nematodes per plant and promoted plant growth.	[44]
Carrot root-knot nematode	<i>Meloidogyne incognita</i>	Antinematode	Chitosan caused a synergistic effect against <i>M. incognita</i> on carrots and could be utilized to manage nematodes as a potential sustainable treatment.	[45]
Rice root-knot	<i>Meloidogyne graminicola</i>	Antinematode	Chitosan reduced root-galling and nematodes development and induced a plant defense mechanism against nematodes.	[46]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
<i>Thymus daenensis</i>	Abiotic stress	Drought stress	Chitosan stimulated osmotic adjustment through proline accumulation and reduction of lipid peroxidase levels, which increased the integrity of cell membranes of thyme leaves.	[47]
Potato plants	Abiotic stress	Drought stress	Chitosan reduced membrane relative permeability and malondialdehyde concentration of potato leaves; raised the concentration of proline and soluble proteins; enhanced the activities of superoxide dismutase, and enhanced their antioxidation ability; increased the activities of protective enzymes and regulated the content of osmotic regulatory substances like peroxidase.	[48]
Apple seedlings	Abiotic stress	Drought stress	Chitosan acted as an exogenous antioxidant that enhanced resistance to oxidative stress during drought in apple seedlings.	[49]
Cowpea plants	Abiotic stress	Drought stress	Chitosan increased the thickness of the midrib region, mesophyll tissue, and the midrib vascular bundle, therefore reduced water stress in cowpea.	[50]
Sweet basil (<i>Ocimum ciliatum</i> , <i>Ocimum basilicum</i>)	Abiotic stress	Drought stress	Chitosan reduced the harmful effect of water stress, increased plant growth parameters, and significant effected on total phenol content and antioxidant activity of the extracts of the two species.	[51]
Coffee plants	Abiotic stress	Drought stress	Chitosan enhanced the content of chlorophyll and carotenoids in the leaves of coffee seedlings, increased mineral uptake and stimulated the growth of coffee seedlings.	[52]
Pepper plants	Abiotic stress	Antitranspirant	Chitosan decreased transpiration due to inducing closure of the plant's stomata by a decrease of K in the guard cells.	[53]
Safflower and Sunflower seedlings	Abiotic stress	Anti-salinity	Chitosan exhibited positive effects on salt stress alleviation through the reduction of MDA and increased proline contents and CAT and POX activities in both crops.	[54]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Wheat seedlings	Abiotic stress	Anti-salinity	Chitosan increased antioxidant enzyme: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities, reduced malondialdehyde (MDA) content in leaves, and accelerated the accumulation of proline.	[55]
Maize plants	Abiotic stress	Anti-salinity	Chitosan ameliorated the effects of salinity stress and improved plant growth.	[56]
Rice seedlings	Abiotic stress	Anti-salinity	Chitosan lowered malondialdehyde and increased proline levels and enhanced the activities of catalase and peroxidase enzymes.	[57]
<i>Fenugreek (Trigonella foenum-graecum)</i>	Abiotic stress	Anti-salinity	Chitosan increased plant dry weight, length of stem and roots, leaf relative water content, and amount of anthocyanin photosynthetic pigments, resulting in better growth and establishment of fenugreek plants.	[58]
Bean (<i>Phaseolus vulgaris</i>)	Abiotic stress	Anti-heat stress	Chitosan enhanced uptake of mineral nutrients, cell division, and chlorophyll accumulation in the leaves.	[59]
Cucumber seedlings	Abiotic stress	Anti-heat stress	Chitosan improved the cold resistance of cucumber seedlings, protected the membrane system, improved the capability of eliminating active oxygen species, and alleviated the damage to photosynthetic organization.	[60]
Maize plants	Abiotic stress	Anti-heat stress	Chitosan caused a decline in malondialdehyde (MDA) content and relative permeability of the plasma membrane. Also, increased concentrations of soluble sugars, proline, and peroxidase (POD) and catalase (CAT) activities led to an enhanced germination index and reduced the mean germination time (MGT). Additionally, it increased shoot height, root length, and shoot and root dry weights.	[61]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Bean (<i>Phaseolus vulgaris</i>)	Plant improvement	Promote plant growth	Chitosan enhanced shoot and root length, fresh and dry weights of shoots and roots, and leaf area in beans.	[62]
Rice and soybean plants	Plant improvement	Promote plant growth	Chitosan promoted plant length and shoot growth in both rice and soybean.	[63]
Cucumber plants	Plant improvement	Promote plant growth	Chitosan increased the vegetative growth and increased the total yield of cucumber fruits in terms of quantity and quality.	[64]
Okra plants	Plant improvement	Promote plant growth	Chitosan enhanced the biochemical activities in okra, resulting in an increase in growth and the fruit-bearing nodes, and improved the yield quality and quantity.	[65]
Tomato and eggplant plants	Plant improvement	Promote plant growth	Chitosan enhanced the growth and functional components of tomatoes and eggplants by increasing plant height, branch, leaf, flower, and fruit number and size.	[66]
<i>Verbena bonariensis</i>	Plant improvement	Promote plant growth	Chitosan enhanced the plant height, number of inflorescences and number of leaves.	[67]
<i>Eustoma grandiflorum</i>	Plant improvement	Promote plant growth	Chitosan soil treatment resulted in greater shoot length, stem diameter, weight of cut flowers, and an increase in the number of flowers.	[68]
Soybean leaves	Plant improvement	Stimulate plant defense responses	Chitosan increased activity of phenylalanine ammonia-lyase and tyrosine ammonia-lyase enzymes which increased total phenolic content in plants.	[69]
Chickpea plants	Plant improvement	Imparts immunity against Fusarium	Chitosan caused significant ECM and guard cell remodelling, and translated ECM cues into cell fate decisions during fusariosis.	[70]
Several plants	Plant improvement	Eliciting plant immunity	Chitosan-induced defense responses such as increased cytosolic H ⁺ and Ca ²⁺ , MAP-kinase activation, callose apposition, oxidative burst, and hypersensitive response (HR) Moreover, it induced the synthesis of abscisic acid, jasmonate, phytoalexins, and pathogenesis-related (PR) proteins.	[71]

Host/disease name	Pathogen/stress	Chitosan effects	Chitosan mode of action	References
Soybean cells	Plant improvement	Elicited callose synthesis	Chitosan caused the rapid and quantitative formation of callose and elicited other metabolic changes that appeared to be important for resistance against pathogens in soybean cells.	[72]

Table 1.
Chitosan mechanisms in plant protection against biotic and abiotic stresses.

fatty acid-rich membranes such as palmitic acid or stearic acid (low fluidity membrane) [15]. The incubation period affects the antifungal activities of chitosan. The growth inhibition of *F. oxysporum* f. sp. *radicis-lycopersici* at a low concentration of chitosan increases with the long incubation period [74]. Chitosan causes excessive mycelial branching, abnormal shapes, swelling and hyphae size reduction in *F. oxysporum* f. sp. *cubense* [27], *F. solani* f. sp. *glycines* [28], *Botrytis cinerea*, and *A. alternata* [24]. Chitosan is also responsible for cytological alteration, protoplasm dissolution, and large vesicles of fungus [27]. Chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea* and *F. oxysporum* f. sp. *albedinis* [30, 75]. Fungi that have been exposed to chitosan make fewer spores than untreated fungi. In other instances, full sporulation suppression was observed following chitosan therapy. The length and shape of the conidia of *Ranunculus stolonifer*, *Penicillium digitatum*, and *F. oxysporum* were considerably influenced by chitosan [76]. The application of chitosan against *F. oxysporum* f. sp. *cucumerinum* showed a significant decrease in wilt disease severity compared to chitosan-untreated Fusarium [31].

3.2 Antibacterial activity

Gram-positive and gram-negative bacteria have significantly different cell wall structures and surface polarity, which results in different sensitivity to chitosan [13]. The cell wall of gram-negative bacteria is distinguished by the presence of lipopolysaccharides, which contain phosphate and pyrophosphate groups [77]. This provides it with a high negative charge, making it more bound to chitosan [78]. While the cell wall of gram-positive bacteria contains polysaccharides associated with lipoteichoic and teichoic acids. Teichoic acid is negatively charged due to the presence of phosphate groups in its structure which gives it a small negative charge that makes it less bound to chitosan. The stopping of the teichoic acid biosynthesis pathway in *Staphylococcus aureus*, led to an increase in chitosan resistance [35]. Gram-negative bacteria are more hydrophilic than gram-positive bacteria and also have a thinner cell membrane [79]. This explains why gram-negative bacteria react to chitosan differently from gram-positive bacteria (**Figure 2**).

Chitosan has potent antibacterial activities against a variety of plant pathogenic bacteria like *S. aureus* [80], *Streptomyces scabies* [36] *Ralstonia solanacearum* [81], *Xanthomonas* spp. [26, 37], *Pseudomonas* spp. [38, 80], and *Acidovorax* spp. [39, 40]. The inhibitory activity of chitosan against bacteria varied with molecular weight [82], concentration [39], solvent type [29], bacterial type (gram-positive/gram-negative) [83], cell wall structure [84], period of incubation and abiotic factors [85]. Chitosan binds to the negatively charged surface of bacteria at low concentrations (less than

0.2 mg/ml) to cause agglutination, but at higher concentrations, the presence of more chitosan positive charges may have given the bacteria a net positive charge that keeps them suspended [86]. Moreover, Goy et al. [87] suggest that chitosan is responsible for the hydrolysis of peptidoglycans, the main component of the bacterial cell wall, increasing electrolyte leakage, and potentially causing the death of the plant pathogens. Additionally, Liang et al. [88] reported that chitosan is the responsible substance for the destruction of the bacterial cell membrane, which causes death due to the leakage of intracellular substances. Chitosan applied to tomato plants inhibited the growth of *Xanthomonas vesicatoria* [26]. Chitosan-protected cucumber from *Pseudomonas syringae* pv. *lachrymans* that causes bacterial angular leaf spot damage [89]. Chitosan has decreased the disease incidence of broccoli that was infected with *Pseudomonas fluorescens* [38]. The disease index of watermelon seedlings infected with *Acidovorax citrulli* was significantly reduced by chitosan at 0.4 mg/ml [40]. Chitosan solution at 0.10 mg/ml markedly decreased the surviving cell number of *Xanthomonas* pathogenic bacteria isolated from different geographical origins compared with the control after 6 h of incubation, regardless of the bacterial strain [90].

3.3 Antiviral activity

One of the most destructive plant diseases is the viral disease which causes serious damage to many plant species, affecting agroecosystems and food security. For that reason, searching for new eco-friendly application technologies to suppress the invasion of viral plant diseases is urgently needed to fulfill the nutrients required to feed the world's population [91]. Chitosan and its derivatives have been used as a promising and powerful tool against plant viruses. Chitosan has demonstrated antiviral activity against Potato virus X (PVX) through the possible mechanism of induced resistance and responsive defense mechanisms or the inhibition of systemic propagation of plant viruses in potato plants [4]. Complete inhibition or suppression of systemic virus multiplication in the host plant has not clearly proven the capability of chitosan to stop the virus activation. However, the multiplication block may be due to the binding of chitosan molecules with the nucleic acid of a targeted virus, causing serious damage to the viral genome [92]. Studies by Jia et al. [41] explained the role of chitosan in inducing systemic acquired resistance in Arabidopsis plants infected with Tobacco mosaic virus (TMV) and which signaling pathways are involved in the processes of defense mechanisms. Their obtained results revealed that chitosan application induced TMV resistance through specific pathways in the plant. The induction in the Arabidopsis plants happened through the jasmonic acid pathway-deficient (Arabidopsis plants *jar1*) and at the same time did not induce the salicylic acid pathway-deficient (Arabidopsis plants *NahG*). The application of chitosan as a protective and curative treatment against Alfalfa mosaic virus (AMV) on *Nicotiana glutinosa* plants under greenhouse conditions was studied by [42]. They proved that the AMV concentration was significantly reduced through both protective and curative treatment with 70.43% and 61.65%, respectively. On the other hand, possible ways of inducing systemic resistance and responsive defense mechanisms were measured, resulting in an increase of total phenols and carbohydrates as well as phenylalanine ammonia-lyase (PAL) and peroxidase.

3.4 Antinematode activity

Serious, highly damaging, and economic losses to a wide range of plant hosts, including fruit trees, vegetables, agronomic crops, foliage crops, grasses, nuts, and

forest trees, were reported to be caused by several nematode species under different genera. Economic losses to more than 2000 kinds of higher plant species are often great when certain plant species are grown in warm regions around the world. The development of efficient new and eco-friendly nematode management strategies such as biological control, natural products, plant extracts, and botanical products is needed urgently to reduce the high toxicity of chemical nematicides [93]. Studies by El-Ansary et al. [43] revealed that chitosan significantly reduced the disease severity of root-knot nematode infection caused by *Meloidogyne incognita* in banana plants cv. Williams with an improvement in plant growth parameters and yield production. Chitosan-treated tomato plants produced less root-knot nematode reproduction, which enhanced the size, weight, and growth of the plant's roots and shoots [44]. Recently, Khan et al. [45] evaluated chitosan as a nematicide against the infestation caused by *Megalaima incognita* in carrot plants under *in vivo* and *in vitro* conditions. They reported that egg masses and second-stage juvenile (J2s) of *M. incognita* were affected by the usage of different concentrations of 500, 1000, 1500, 2000, and 2500 ppm of chitosan. Maximum mortality of J2s and the highest inhibition in egg hatching was observed at 2500 ppm of chitosan after 36 h incubation period.

4. Role of chitosan in plant protection against abiotic stress

Chitosan improves plant tolerance to drought, salinity, and high temperature [94] (Table 1).

4.1 Effect on drought stress

Agricultural productivity is limited by drought stress, which has a number of negative effects on plant health and lowers plant growth and yield. Chitosan is one of the effective solutions to mitigate the harmful effects of drought stress on plants (Figure 3a). Under extreme drought stress, the free proline content in leaves considerably increases [95]. In chitosan-treated plants, the accumulation of proline production in the absence of drought stress was enhanced [96]. After chitosan treatment, proline accumulation was enhanced in the thyme plants [47]. The accumulation of proline helps in reducing the leaf water potential, improves the turgor of leaves, and facilitates water delivery to them. In addition, proline is crucial for maintaining redox balance, quenching ROS, and osmotic adjustment [97].

On the other hand, a water deficit condition disturbs the plant's cell membrane integrity. The membrane permeability and malondialdehyde concentration are related to each other, indicating membrane stability. Malondialdehyde levels rise in conditions of water deficiency; this lipid peroxidation byproduct has the potential to lead to membrane leakage because of the accumulation of free radicals. Chitosan functions as a positive regulator in osmotic adjustment and can eliminate the adverse effects of drought stress symptoms by decreasing the production of lipid peroxidation. The pre-treatment of thyme and potatoes with chitosan reduced lipid peroxidation, removed ROS, and improved cell membrane integrity [47, 48]. In apple seedling leaves exposed to drought stress, chitosan significantly improved the integrity of cell membranes and decreased the production of malondialdehyde and electrolyte leakage [49]. Plants attempt to reduce the harmful effect of drought by raising the level of soluble sugar in the cell by breaking down the polysaccharides that help in the preservation of turgor [98]. Chitosan is an important source of sugars, e.g., glucose, fructose, trehalose,

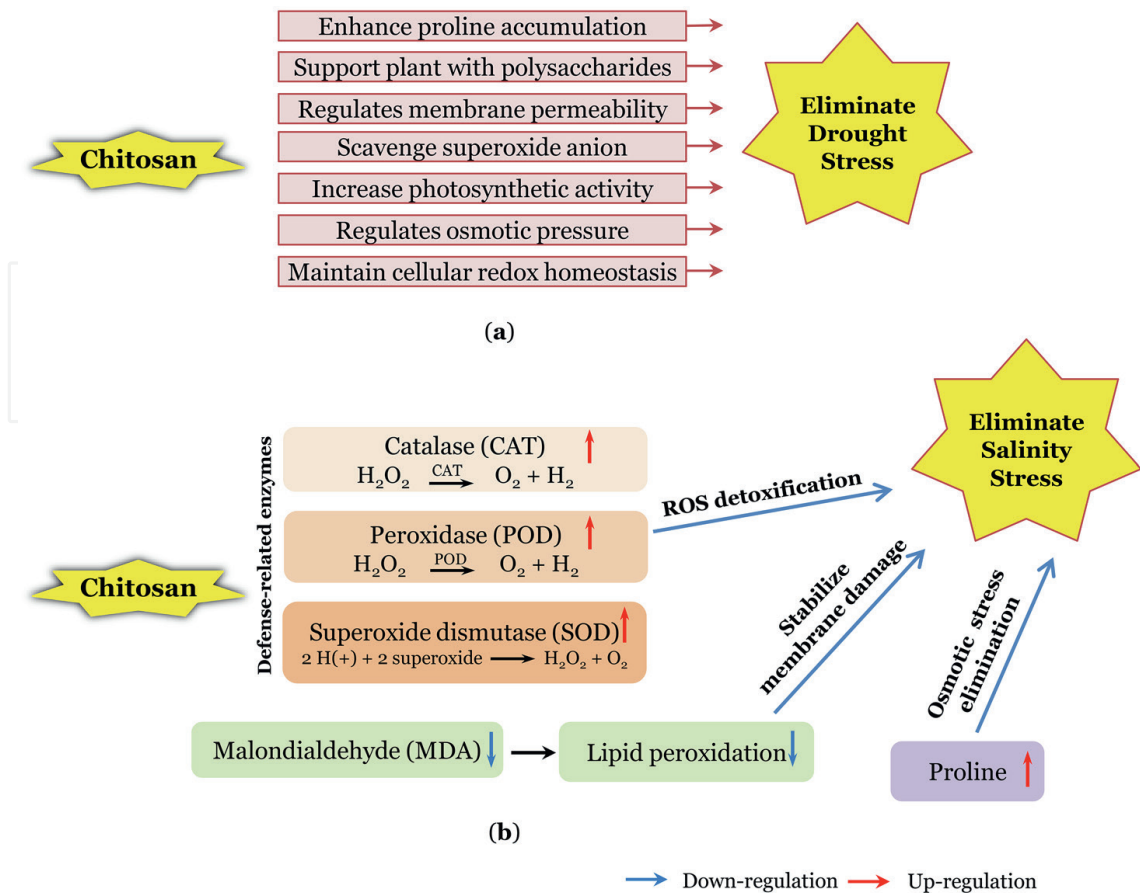


Figure 3. Role of chitosan in removing (a) drought stress and (b) salinity stress.

sorbitol, mannose, and myoinositol that plants need to overcome drought [96]. These might enhance osmotic adjustment and maintain carbon balance in response to dehydration stress, which would improve drought resistance.

Additionally, drought stress hinders photosynthetic activity by reducing chlorophyll production. This could be caused by oxidative damage to chloroplast lipids, pigments, and proteins or by the loss of chlorophyll pigment complexes or light-harvesting protein complexes [50, 99]. Chitosan spraying resulted in an increase in chlorophyll and total carbohydrates which increased photosynthesis levels in soybean and maize [100], cowpea [50], and bean [62]. This might be the result of higher nitrogen and potassium levels in plant shoots, which aid in raising the number of chloroplasts per cell and boosting chlorophyll synthesis. Additionally, chitosan treatment's release of amino compounds with a higher availability level encourages the synthesis of chlorophyll [63].

Commercial antiperspirants are better than chitosan in raising the efficiency of the plant in retaining water, but they reduce the photosynthetic rates and carbon uptake as a result of reducing the internal CO_2 in leaves [71]. The efficiency of chitosan as an antiperspirant is due to its control of the mechanism of opening and closing the stomata, which allows the entry of carbon from the atmosphere into the plant. Thus, maintaining the efficiency of the photosynthesis process inside the plant, unlike the commercial antitranspirant, this acts as a thin antitranspirant membrane that covers the leaves' surface and blocks stomata, which prevents the entry of carbon needed for photosynthesis. Chitosan use as an antitranspirant substance would be more suitable for plants that experience occasional drought occurrences. Chitosan-treated plants

would enable their natural physiological system to quickly recover maximum carbon uptake while sustaining biomass and yield in these circumstances [53]. Histological examination of chickpea leaves revealed stomatal closure accompanied by decreases in stomatal conductance and transpiration rate in chitosan-treated seedlings during *Fusarium* infection, indicating the presence of stomatal immunity associated with chitosan [70].

4.2 Effect on salinity stress

Salinity can prevent plants from absorbing water and nutrients due to low external osmotic potential. In addition, the direct ionic effect results in excessive accumulation of Na and Cl ions, which causes toxic effects, closes the stomata, lowers internal CO₂, and decreases the rate of photosynthesis. Salt-induced stress conditions were discovered to have lipid peroxidation brought on by the buildup of malondialdehyde [101]. A decrease in malondialdehyde content after chitosan treatment stabilized membrane damage and may have given salt stress tolerance [54]. Plants have developed their own inherent ROS scavenging mechanism by producing antioxidant enzymatic compounds, e.g., superoxide dismutase, peroxidase, and catalase; increased abundance of these enzymes denotes effective ROS detoxification (**Figure 3b**). These enzymes were shown to be elevated in chitosan-treated plants, and they are crucial for reducing salt stress since they are stronger antioxidant enzymes [54, 55]. Chitosan has the ability to scavenge superoxide anions due to the presence of hydroxyl and amino groups that react with ROS [102]. Chitosan treatment reduces malondialdehyde levels and increases antioxidant enzyme activities during salinity stress, which minimizes the negative effects of salt stress on maize [56]. The low concentration of chitosan treatment could counteract the harmful effects of salt stress. A small amount of chitosan applied to sunflower seeds can suppress enzyme activity and decrease the oxidative damage brought on by salt stress [54]. During salt stress, wheat seed treated with chitosan showed increased levels of the antioxidant enzymes (superoxide dismutase, peroxidase, and catalase), stomatal conductance, and photosynthetic rate [55]. When a plant is exposed to salt stress, its chlorophyll concentration drastically decreases because of the accumulation of chlorophyllase and the instability of protein complexes. In salt stress, proline levels were elevated, which might be the result of increased proline biosynthesis, decreased proline oxidation to glutamate, or decreased utilization of synthesized proline. Plants are mostly protected against osmotic stress by proline [103]. Proline levels were shown to increase as chitosan concentrations increased [54].

4.3 Effect on heat stress

There is a paucity of published data on the use of chitosan in heat stress. Heat stress is frequently viewed as a complex issue because it frequently occurs in conjunction with drought stress and is challenging to measure [57]. Foliar application of chitosan combined with humic acid and zinc is the best treatment that could be recommended for dry bean production to withstand heat stress [59]. Chitosan use could reduce the effects of high temperatures by promoting abscisic acid activity [53]. According to Choi et al. [104], abscisic acid can activate genes associated with heat shock, such as ABF3, whose overexpression may improve heat stress tolerance. Applying chitosan to cucumber leaves at low temperatures can improve their proline and soluble protein levels as well as the activity of antioxidant enzymes [60].

5. The role of chitosan in enhancing plant traits

When a pathogen attacks a plant, a coordinated signaling mechanism is induced which leads to the accumulation of several gene products. Once the pathogen gets recognized by plant receptors, rapid localized cell death will be developed, which is known as the hypersensitive response, causing necrosis at the site of infection. While in the sections of the plants that are not infected, a systemic expression of a broad spectrum of resistance will be induced to prevent additional pathogen infection. Then reactive oxygen species are produced, defense-related genes are activated, and the expression of genes that produce compounds, including terpenes, phytoalexins, defense enzymes, and pathogenesis-related proteins, is increased.

Chitosan can induce plant resistance and activate several defense processes in plant tissue [32]. These defense mechanisms include accumulation of hydrolytic enzymes, synthesis of proteinase inhibitors and pathogenesis-related (PR) proteins, enhancement of phytoalexins, formation of callose, promotion of lignification, and induction of reactive oxygen species (ROS) (**Figure 4**) [46, 105].

5.1 Plant growth promotion

Chitosan promotes plant growth in a variety of crops by significantly influencing the development rates of shoots, roots, flowering, and the number of flowers. Due to the great hydrophilicity of chitosan molecules, it adjusts the osmotic pressure in plant cells by increasing the absorption of water and important nutrients [61] and minimizing stress damage in plant cells. Chitosan increases the efficiency of plant nitrogen uptake, acts as a source of energy and an additional carbon source for the synthesis of carbohydrates, and acts as an activator for various metabolic processes [14, 51]. Chitosan encourages the proliferation of root cells by activating auxin and cytokinin, which further boosts nutrient uptake [52]. Chitosan assisted in triggering the hydrolytic enzymes required for the mobilization and degradation of reserve food components, including protein and starch [106]. Significant growth enhancements have been recorded after chitosan application by several studies in artichoke [107], cucumber [64], okra, [65], eggplant and tomato [66], strawberry [108], potato [109], chili [33], and watermelon [110]. Chitosan can enhance plant physiological mechanisms, e.g., nutrient absorption, cell elongation, cell division, enzymatic activation, and protein synthesis [111].

5.2 Increase photosynthetic activity

Chitosan improves the photosynthesis process by enhancing stomatal function and reducing the breakdown of chlorophyll [112]. Chitosan increased the chlorophyll levels in leaves by 13.4% compared to control plants [67]. The application of chitosan improved chlorophyll content and plant productivity in chili [113], peanut, and coffee plants [52]. The use of chitosan protects chlorophyll content in stressful circumstances; it increases the chlorophyll content in fenugreek under a salinity conditions [58]. Applying chitosan to cucumber leaves at low temperatures can improve their proline and soluble protein levels as well as the activity of antioxidant enzymes [60].

5.3 Up-regulate pathogenesis-related proteins

Plants produce proteins known as pathogenesis-related proteins (PR) in response to a pathogen invasion. They are induced as a part of systemic acquired resistance

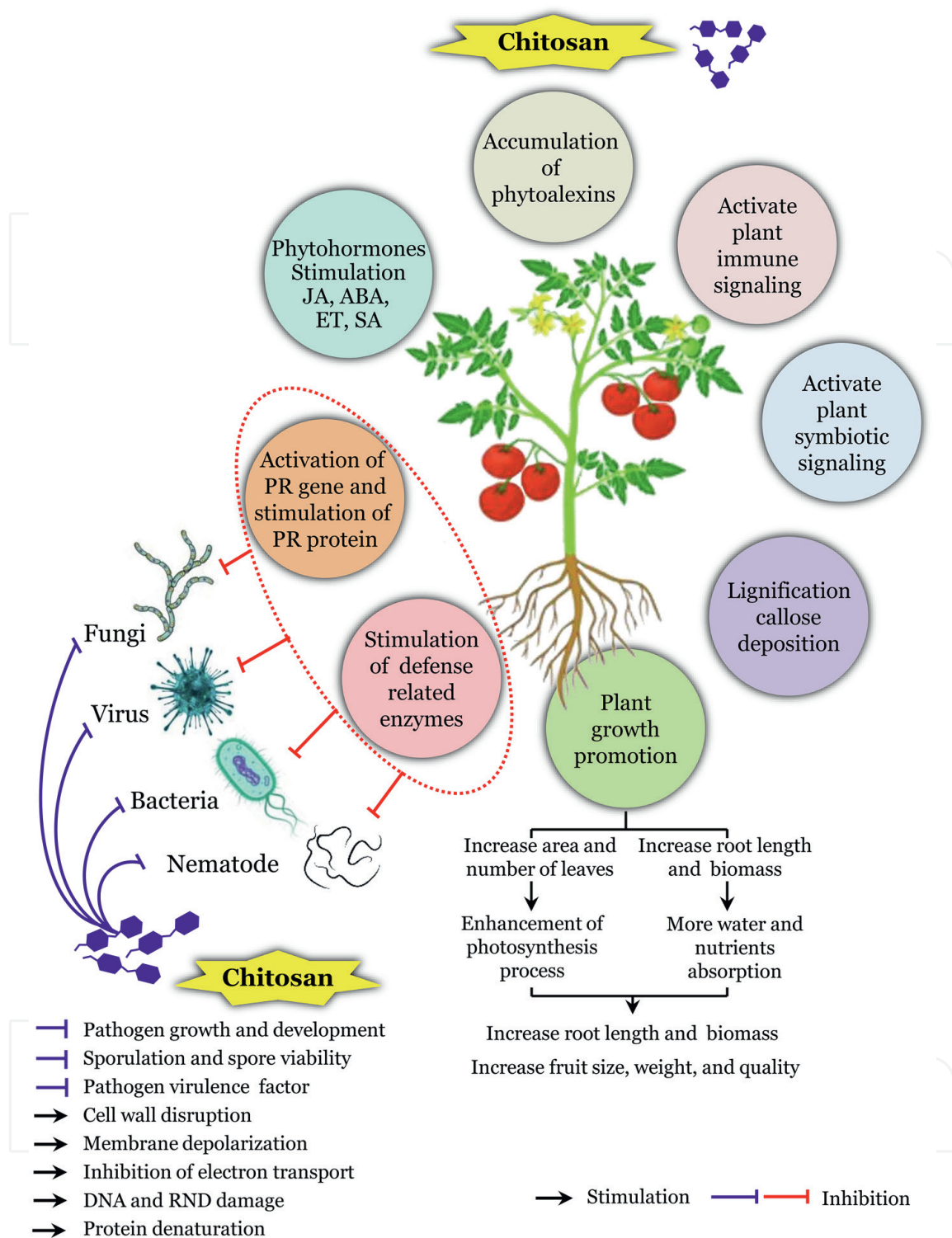


Figure 4. Mechanisms of chitosan in the enhancement of plant defense system and inhibition of phytopathogens.

where their corresponding genes are activated by infections. Chitosan has been characterized as an elicitor that causes plants to create a wide variety of pathogenesis-related proteins with antimicrobial action. Among these pathogenesis-related proteins are chitinase and 1,3-glucanase, two hydrolytic enzymes that destroy pathogen cell walls that contain chitin and/or β -D-glucans as major structural components [114].

Chitosan appeared to use a variety of mechanisms to enhance pathogenesis-related gene function, including activating membrane receptors and altering the DNA

structure of the plant. Chitosan of low molecular weight was more effective at inducing the defense-related genes β -1,3-glucanase and chitinase than the higher molecular weight [115]. Chitosan triggered the transcriptional up-regulation of defense-related genes β -1,3-glucanase and chitinase in rice seedlings [115]. Chitosan significantly increased the expression of general defense response genes in oat leaves [116]. Chitosan was able to promote resistance in pears and peaches by increasing chitinase and β -1,3-glucanase activities [74, 117]. Chitosan has been proven to be effective at triggering plant defense mechanisms by increasing the level of β -1,3 glucanase and chitinase enzymes in strawberry and pepper plants [118].

5.4 Stimulate defense-related enzymes

Chitosan acts as a physiologic elicitor, enhancing defense-related enzymes, e.g., peroxidase, catalase, superoxide dismutase, polyphenol oxidase, and phenylalanine ammonia-lyase [119]. Peroxidase helps oxidize phenolic and enediolic compounds into quinones and hydrogen peroxide [120]. Peroxidase increases pathogen resistance in plants [121]. The chitosan treatment markedly boosted the peroxidase activity in the flesh surrounding the pear fruit wound [74]. Chitosan induced peroxidase expression activity in date palm roots when injected at three concentrations (0.1, 0.5, and 1 mg/ml) [30]. Fruit treated with chitosan maintained relatively higher peroxidase gene expression than control fruit [117]. Catalase is crucial for plant senescence and defense [122], it transforms H_2O_2 into H_2O and O_2 . Catalase activity was increased in the chilling-sensitive and chilling-tolerant maize seedlings after chitosan treatment [61]. Superoxide dismutase destroys radicals and protects cells against the effects of oxidative stress. Superoxide dismutase activity has increased after chitosan treatment of *Hydrilla verticillata* [123]. Drought stress decreased the activities of the antioxidant enzymes catalase and superoxide dismutase in apple leaf tissues, but chitosan treatment enhanced their activities [49].

Polyphenol oxidase participates in plant defense by encouraging the production of lignin, which strengthens the cell wall structure and deters disease penetration [34]. Chitosan has significant antibacterial efficacy against rice leaf streak and leaf blight produced by *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae*, respectively, by increasing polyphenol oxidase activity [37]. Phenylalanine ammonia-lyase transforms L-phenylalanine to ammonia and trans-cinnamic acid [124]. It is induced in host tissues as a result of pathogen infection [69]. In grape berries, rice, and wheat, elicitation with chitosan resulted in an increase in phenylalanine ammonia-lyase [37]. When chitosan was injected into the roots of date palm, it enhanced the essential components of the host resistance against *F. oxysporum* f. sp. *albedinis*, increased the level of phenolic compounds, and stimulated date palm peroxidase and polyphenol oxidase activities [30].

5.5 Induce signal regulation

5.5.1 Activation of signal transduction

Chitosan can trigger the plant's defense mechanisms and functions as a regulatory molecule in signal transduction through several signaling pathways. When chitosan activates a particular receptor located on the cell membrane or intracellular, one or more second messengers relays the signal to the cell. This triggers a range of physiological responses as a single signal can be amplified

and develop a complex signaling network. This is called signal transduction. The chitosan-mediated signal pathway includes reactive oxygen species (ROS), Ca^{2+} , nitric oxide (NO), salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), and ethylene (ET) [125].

One of the first reactions to a microbial pathogen attack is the oxidative burst, which has been demonstrated to occur upon chitosan elicitation. It is characterized by the quick and temporary creation of enormous levels of ROS (hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), singlet oxygen ($^1\text{O}_2$), and superoxide (O^{-2}) (**Figure 5**) [126]. Chitosan induced the accumulation of H_2O_2 in tomatoes [127]. Upon pathogen infection, one of the quickest responses is an increase in cytosolic Ca^{2+} [128]. Within 20 min of being treated with chitosan, Glycine max suspension-cultured cells began to synthesize callose. However, in the absence of exogenous Ca^{2+} , chitosan-induced callose production was not achievable [72]. Chitosan increased the amount of free cytosolic Ca^{2+} in *Arabidopsis* and stomatal closure [129].

NO, another messenger, implicated in the plant defense response against pathogens and involved in chitosan-induced resistance [114]. Downy mildew-infected pearl millet seedlings treated with chitosan exhibited increased NO buildup commencing 2 h after inoculation, as well as protection from downy mildew [114]. Chitosan induced the generation of NO and phosphatidic acid in tomato cell culture while the phospholipase-mediated signaling pathway was inhibited after using NO scavenger, indicating that the production of phosphatidic acid during the plant defensive response needed NO [130].

Plant hormones regulate growth processes in plants and play important roles in plant responses to biotic and abiotic stresses [131]. Salicylic acid, jasmonic acid, and ethylene play a crucial role as signaling molecules in modulating *Arabidopsis*' responses to biotic and abiotic stress. Jasmonic acid and ethylene are central signaling molecules

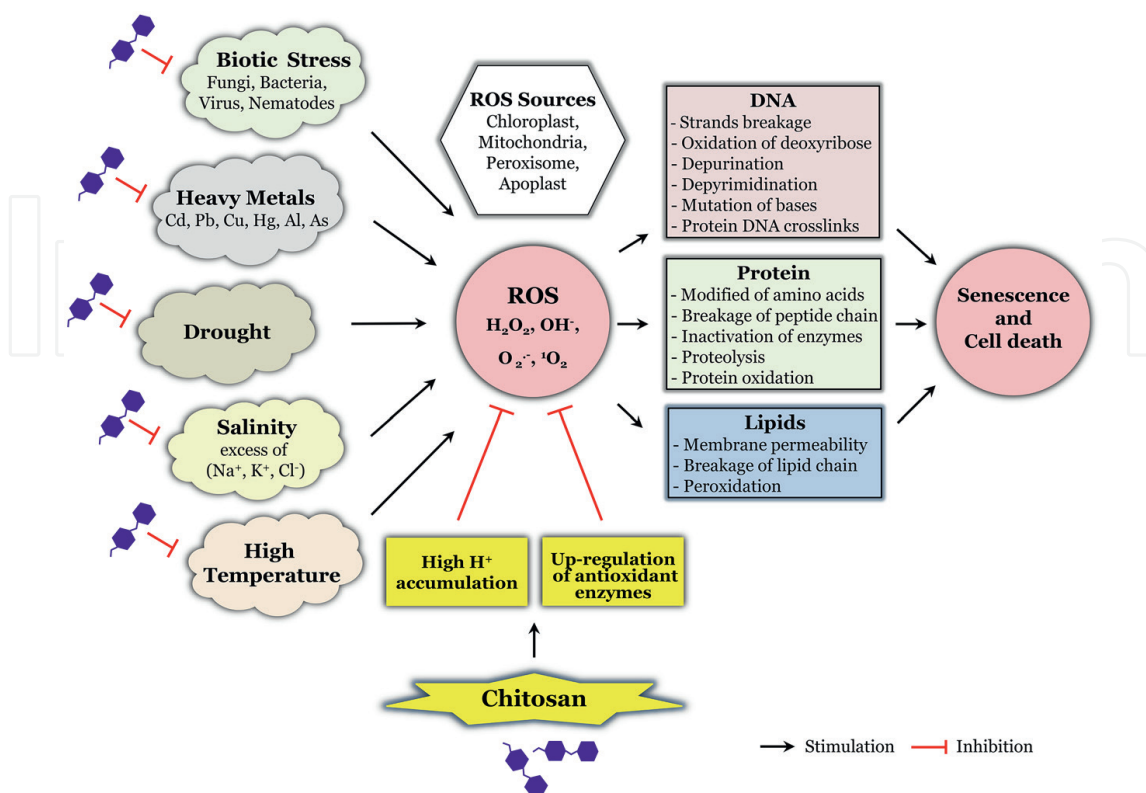


Figure 5. An overview of how chitosan helps plants withstand phytopathogens, abiotic stress, and ROS.

in the induced systemic resistance; on the other hand, salicylic acid is involved in systemic acquired resistance, which occurs when a pathogenic attack on one area of the plant results in resistance in other parts. It has been suggested that jasmonic acid is a component of a signal transduction pathway that controls the activation of genes involved in plant defensive responses to pathogen invasion. Chitosan significantly increased the jasmonic acid in wounded rice leaves [132]. ABA regulates the intensity and speed of callose deposition. Additionally, ABA-mediated signaling transduction is crucial for plants to respond to abiotic and biotic stresses [133]. Chitosan activated the defense signaling pathways in tomato plants against *A. solani* and *X. vesicatoria* [26].

5.5.2 Activation of symbiotic signaling

Beneficial microorganisms are able to form a symbiotic connection while living in close proximity to their plant hosts, which aids in the acquisition of nutrients necessary for plant growth. Additionally, the plant meets some of the requirements that helpful microorganisms need to complete their metabolic processes for growth and reproduction [134]. Chitosan triggered symbiotic signaling between plants and beneficial microbes [135]. Lipo-chitoooligosaccharides, which are chitin oligosaccharides bound to a lipid moiety, have been discovered to be released by mycorrhizal fungi and rhizobium bacteria during the development of symbiotic interactions and have been recognized as key signaling molecules triggering the plant symbiotic response [136]. Chitosan undergoes enzymatic degradation without harming the beneficial rhizosphere biota in the soil and promotes symbiotic interactions between plants and microorganisms, causing changes in the rhizosphere's microbial balance and harming plant diseases [44].

5.6 Usage as bio-fertilizer

As a result of the repeated use of inorganic fertilizers that are difficult to decompose, the toxicity of the soil increased, which affected the beneficial microorganisms present in the soil and the properties of the soil. Therefore, the use of chitosan at low concentrations as a bio-fertilizer was a safe and effective alternative to avoid the risks of using inorganic fertilizers. Utilizing chitosan as a biofertilizer showed a significant decrease in late blight infestation of potato tubers and an increase in plant nutrient uptake [137]. The addition of chitosan to soil improved the phosphorous and nitrogen content in *Eustoma grandiflorum* [68].

Large amounts of inorganic fertilizers are lost in the soil due to the inability of plants to absorb them, causing farmers to over-apply them, resulting in their presence in the soil at higher than required rates, causing soil toxicity, water pollution, and damage to crops, particularly vegetables, which are severely affected by fertilizer toxicity [138]. However, when chitosan coating on fertilizer is added to the soil, it improves the absorption of inorganic fertilizer by plants, which minimizes the use of fertilizers, makes the soil less toxic, and reduces the production cost [139].

6. Recovery of contaminated agricultural wastewater and soil

The concentration of heavy metals and other contaminants in the environment is increasing rapidly as a result of multiple human activities. Heavy metals are

dangerous because they are highly poisonous, do not biodegrade, and cause cancer and other disorders. Therefore, it is necessary to find an efficient way to remove them from the environment and dispose of them. From this perspective, bio adsorption is acknowledged as an affordable and effective solution [140]. Effective pollutant removal makes it possible to reuse valuable resources like cultivable soil and fresh water. The application of chitosan-based adsorbents in these areas has been extensively studied due to their low production costs, biocompatible and biodegradable nature, strong resistance to antimicrobial attack, and absence of the creation of potentially toxic secondary end products [140]. The chemical composition of chitosan makes it simple to combine with certain ions, molecules, and other compounds to produce complex structures for specific applications. Carboxylated graphene oxide-chitosan (GO-COOH/CS) spheres were used for the immobilization of Cu^{2+} from water and soil, as well as for reducing the bioaccumulation of Cu^{2+} from wheat plants [141]. Cesium-contaminated clay can be cleaned using ionized chitosan and magnetic microgels functionalized with Prussian blue analogs, 200 mg/g of ionized chitosan hydrochloride can achieve 87.6% cesium release from clay in about 2 h [142].

A combination of chitosan and duckweed was assessed for its ability to remove boron from water. Chitosan beads had the maximum boron absorption capacity of chitosan at 3.18 mg/g [143]. Chitosan can help reduce the environmental effects of industrial wastewater treatments and soil acidity by reducing CO_2 and SO_2 [144]. Columns packed with chitosan have the ability to remove arsenic from groundwater [145]. A composite consisting of chitosan and hyacinth extract effectively absorbs Cu, Pb, and Cd ions from water [146]. Cd removal from the soil and aquatic environments was examined using the magnesium oxide biochar-chitosan composite (MgO-BCR-W) [147].

The adsorbent made of chitosan/ MnO_2 nanocomposite was employed to extract Cr (VI) from the aqueous solutions [148]. A magnetic chitosan/polyacrylic acid nanocomposite successfully adsorbed Pb (II) from an aqueous solution [149]. Iron chitosan microspheres were synthesized by ionotropic gelation for the removal of arsenic (V) from water [150]. Chitosan contains functional amino and hydroxyl groups, which enables it to form compounds with heavy metals. Foliar application of chitosan can reduce the harmful effects of cadmium in *Brassica rapa* L. [151]. Herbicides can be made more effective by adding chitosan to formulations, which lowers the amount utilized and the risk of hazard accumulation in the environment. A formulation consisting of chitosan and glyphosate exhibits lower phytotoxicity and higher herbicidal efficacy and releases active substances better than using glyphosate alone [152]. Chitosan and tripolyphosphate nanoparticles are efficient carriers to reduce soil sorption, cytotoxicity, and mutagenicity of paraquat and enhance their herbicide activity [153].

7. Chitosan future prospect

Despite extensive research on chitosan, the mechanism of action of chitosan in regulating plant immunity and suppressing the pathogen has not been sufficiently elucidated. It is thought that the mode of action of chitosan may be more complex and involve a series of overlapping details that need to be further studied in the future. Proteomics is one of the contemporary disciplines that has been successfully used to investigate the global variations in protein expression in biological organisms under a variety of environmental conditions [154]. There are a lot of proteomic studies that explain the chitosan mode of action in withstanding biotic

and abiotic stresses in plants. The inhibitory effect of chitosan on *P. expansum* was proteomic analyzed, and 26 proteins were identified and grouped according to their potential biological roles [155]. A comprehensive proteomic study of chitosan-responsive proteins explained the inhibitory mechanism of chitosan against *F. oxysporum* f. sp. *cucumerinum*. This led to the identification of 62 expressed proteins involved in the hindering of the Fusarium cell wall, disrupting DNA, and disrupting structural and functional protein biosynthesis and explained how chitosan influences metabolic pathways [31]. We wish plants and pathogens treated with chitosan would receive abundant proteomic studies in order to make maximum use of chitosan in sustainable agriculture.

Acknowledgements

The authors would like to thank the staff members of Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Conflict of interest

The authors declare no conflict of interest.


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References

- [1] No HK, Park NY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*. 2002;**74**(1-2):65-72. DOI: 10.1016/S0168-1605(01)00717-6
- [2] Ghormade V, Pathan E, Deshpande M. Can fungi compete with marine sources for chitosan production? *International Journal of Biological Macromolecules*. 2017;**104**:1415-1421. DOI: 10.1016/j.ijbiomac.2017.01.112
- [3] Mujtaba M, Khawar KM, Camara MC, Carvalho LB, Fraceto LF, Morsi RE, et al. Chitosan-based delivery systems for plants: A brief overview of recent advances and future directions. *International Journal of Biological Macromolecules*. 2020;**154**:683-697. DOI: 10.1016/j.ijbiomac.2020.03.128
- [4] Chirkov SN. The antiviral activity of chitosan (review). *Applied Biochemistry and Microbiology*. 2002;**38**:1-8. DOI: 10.1023/A:1013206517442
- [5] Kulikov SN, Chirkov SN, Il'ina AV, Lopatin SA, Varlamov VP. Effect of the molecular weight of chitosan on its antiviral activity in plants. *Applied Biochemistry and Microbiology*. 2006;**42**(2):200-203. DOI: 10.1134/S0003683806020165
- [6] Zhao J, Pan L, Zhou M, Yang Z, Meng Y, Zhang X. Comparative physiological and transcriptomic analyses reveal mechanisms of improved osmotic stress tolerance in annual ryegrass by exogenous chitosan. *Genes*. 2019;**10**(11):853. DOI: 10.3390/genes10110853
- [7] Ahmed KBM, Khan MMA, Siddiqui H, Jahan A. Chitosan and its oligosaccharides, a promising option for sustainable crop production—A review. *Carbohydrate Polymers*. 2020;**227**:115331. DOI: 10.1016/j.carbpol.2019.115331
- [8] Hudson SM, Jenkins DW. Chitin and Chitosan, *Encyclopedia of Polymer Science and Technology*. NJ: Wiley Interscience; 2001. DOI: 10.1002/0471440264.pst052
- [9] Rahman L, Goswami J. Recent development on physical and biological properties of chitosan-based composite films with natural extracts: A review. *The Journal of Bioactive and Compatible Polymers*. 2021;**36**(3):225-236. DOI: 10.1177/088391152111014218
- [10] Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Progress in Polymer Science*. 2011;**36**:981-1014. DOI: 10.1016/j.progpolymsci.2011.02.001
- [11] Aranaz I, Alcántara AR, Civera MC, Arias C, Elorza B, Heras Caballero A, et al. Chitosan: An overview of its properties and applications. *Polymers-Basel*. 2021;**13**:3256. DOI: 10.3390/polym13193256
- [12] Rinaudo M. Chitin and Chitosan: Properties and applications. *Progress in Polymer Science*. 2006;**31**(7):603-632. DOI: 10.1016/j.progpolymsci.2006.06.001
- [13] Ke CL, Deng FS, Chuang CY, Lin CH. Antimicrobial actions and applications of chitosan. *Polymers*. 2021;**13**(6):904. DOI: 10.3390/polym13060904
- [14] Chakraborty M, Hasanuzzaman M, Rahman M, Khan MAR, Bhowmik P, Mahmud NU, et al. Mechanism of plant growth promotion

and disease suppression by chitosan biopolymer. *Agriculture*. 2020;**10**(12):624. DOI: 10.3390/agriculture10120624

[15] Palma-Guerrero J, Lopez-Jimenez JA, Pérez-Berná AJ, Huang IC, Jansson HB, Salinas J, et al. Membrane fluidity determines sensitivity of filamentous fungi to chitosan. *Molecular Microbiology*. 2010;**75**(4):1021-1032. DOI: 10.1111/j.1365-2958.2009.07039.x

[16] Ma Z, Garrido-Maestu A, Jeong KC. Application, mode of action, and in vivo activity of chitosan and its microand nanoparticles as antimicrobial agents: A review. *Carbohydrate Polymers*. 2017;**176**:257-265. DOI: 10.1016/j.carbpol.2017.08.082

[17] Divya K, Vijayan S, George TK, Jisha MS. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibres and Polymers*. 2017;**18**(2):221-230. DOI: 10.1007/s12221-017-6690-1

[18] Wang X, Du Y, Fan L, Liu H, Hu Y. Chitosan-metal complexes as antimicrobial agent: Synthesis, characterization and structure-activity study. *Polymer Bulletin*. 2005;**55**(1):105-113. DOI: 10.1007/s00289-005-0414-1

[19] Goy RC, Britto DD, Assis OB. A review of the antimicrobial activity of chitosan. *Polímeros*. 2009;**19**:241-247. DOI: F10.1590/S0104-14282009000300013

[20] Yan D, Li Y, Liu Y, Li N, Zhang X, Yan C. Antimicrobial properties of Chitosan and Chitosan derivatives in the treatment of enteric infections. *Mol*. 2021;**26**(23):7136. DOI: 10.3390/molecules26237136

[21] Hosseinejad M, Jafari SM. Evaluation of different factors affecting

antimicrobial properties of chitosan. *International Journal of Biological Macromolecules*. 2016;**85**:467-475. DOI: 10.1016/j.ijbiomac.2016.01.022

[22] Li YC, Sun XJ, Bi Y, Ge YH, Wang Y. Antifungal activity of Chitosan on *fusarium sulphureum* in relation to dry rot of potato tuber. *Agricultural Sciences in China*. 2009;**8**:597-604. DOI: 10.1016/S1671-2927(08)60251-5

[23] Hernandez-Lauzardo AN, Bautista-Banos S, Velazquez-del Valle MG, Mendez-Montecalvo MG, Anchez-Rivera MM, Bello-Perez LA. Antifungal effects of chitosan with different molecular weights on *In vitro* development of *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. *Carbohydrate Polymers*. 2008;**73**:541-547. DOI: 10.1016/j.carbpol.2007.12.020

[24] De Oliveira Jr EN, De Melo IS, Franco TT. Changes in hyphal morphology due to chitosan treatment in some fungal species. *Brazilian Archives of Biology and Technology*. 2012;**55**:637-646. DOI: 10.1590/S1516-89132012000500001

[25] Guo Z, Chen R, Xing R, Liu S, Yu H, et al. Novel derivatives of chitosan and their antifungal activities *in vitro*. *Carbohydrate Research*. 2006;**341**:351-354. DOI: 10.1016/j.carres.2005.11.002

[26] Ramkissoon A, Francis J, Bowrin V, Ramjegathesh R, Ramsuhag A, Jayaraman J. Bio efficacy of a chitosan based elicitor on *A. solani* and *Xanthomonas vesicatoria* infections in tomato under tropical conditions. *The Annals of Applied Biology*. 2016;**169**(2):274-283. DOI: 10.1111/aab.12299

[27] Al-Hetar MY, Zainal Abidin MA, Sariah M, Wong MY. Antifungal activity of chitosan against *fusarium oxysporum*

- f. sp. *Cubense*. Journal of Applied Polymer Science. 2011;**120**:2434-2439. DOI: 10.1002/app.33455
- [28] Prapagdee B, Kotchadat K, Kumsopa A, Visarathanonth N. The role of chitosan in protection of soybean from sudden death syndrome caused by *fusarium solani* f. sp. *glycines*. Bioresource Technology. 2007;**98**:1353-1358. DOI: 10.1016/j.biortech.2006.05.029
- [29] Rabea EI, Steurbaut W. Chemically modified chitosans as antimicrobial agents against some plant pathogenic bacteria and fungi. Plant Protection Science. 2010;**46**(4):149-158. DOI: 10.17221/9/2009-PPS
- [30] El Hassni M, El Hadrami A, Daayf F, El BEA, Hadrami I. Chitosan, antifungal product against *fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. Phytopathologia Mediterranea. 2004;**43**:195-204. DOI: 10.14601/Phytopathol_Mediterr-1743
- [31] Elagamey E, Abdellatef MAE, Arafat MY. Proteomic insights of chitosan mediated inhibition of *Fusarium oxysporum* f. sp. *cucumerinum*. Journal of Proteomics. 2022;**260**:104560. DOI: 10.1016/j.jprot.2022.104560
- [32] Coutinho TC, Ferreira MC, Rosa LH, de Oliveira AM, Júnior ENO. *Penicillium citrinum* and *penicillium mallochii*: New phytopathogens of orange fruit and their control using chitosan. Carbohydrate Polymers. 2020;**234**:115918. DOI: 10.1016/j.carbpol.2020.115918
- [33] Akter J, Jannat R, Hossain MM, Ahmed JU, Rubayet MT. Chitosan for plant growth promotion and disease suppression against anthracnose in chilli. International Journal of Agriculture Environment and Biotechnology. 2018;**3**:806-817. DOI: 10.22161/ijeab/3.3.13
- [34] Li SJ, Zhu TH. Biochemical response and induced resistance against anthracnose (*Colletotrichum camelliae*) of camellia (*Camellia pitardii*) by chitosan oligosaccharide application. Forest Pathology. 2013;**43**:67-76. DOI: 10.1111/j.1439-0329.2012.00797.x
- [35] Raafat D, von Bargaen K, Haas A, Sahl HG. Insights into the mode of action of chitosan as an antibacterial compound. Applied and Environmental Microbiology. 2008;**74**(12):3764-3773. DOI: 10.1128/AEM.00453-08
- [36] Beausejour J, Clermont N, Beaulieu C. Effect of *Streptomyces melanosporofaciens* strain EF-76 and of chitosan on common scab of potato. Plant and Soil. 2003;**256**:463-468. DOI: 10.1023/A:1026177714855
- [37] Li B, Liu B, Shan C, Ibrahim M, Lou Y, Wang Y, et al. Antibacterial activity of two chitosan solutions and their effect on rice bacterial leaf blight and leaf streak. Pest Management Science. 2013;**69**:312-320. DOI: 10.1002/ps.3399
- [38] Li B, Liu B, Su T, Fang Y, Xie G, Wang G, et al. Effect of chitosan solution on the inhibition of *Pseudomonas fluorescens* causing bacterial head rot of broccoli. Plant Pathology Journal. 2010;**26**:189-193. DOI: 10.5423/PPJ.2010.26.2.189
- [39] Yang C, Li B, Ge M, Zhou K, Wang Y, Luo J, et al. Inhibitory effect and mode of action of chitosan solution against rice bacterial brown stripe pathogen *Acidovorax avenae* subsp. *avenae* RS-1. Carbohydrate Research. 2014;**4**(391):48-54. DOI: 10.1016/j.carres.2014.02.025
- [40] Li B, Shi S, Shan C, Zhou Q, Ibrahim M, Wang Y, et al. Effect of chitosan solution on the inhibition of *Acidovorax citrulli* causing bacterial

fruit blotch of watermelon. *Journal of the Science of Food and Agriculture*. 2013b;**93**:1010-1015. DOI: 10.1002/jsfa.5812

[41] Jia X, Meng Q, Zeng H, Wang W, Yin H. Chitosan oligosaccharide induces resistance to tobacco mosaic virus in *Arabidopsis* via the salicylic acid-mediated signaling pathway. *Scientific Reports*. 2016;**18**(6):26144. DOI: 10.1038/srep26144

[42] Abdelkhalek A, Qari SH, Abu-Saied MAA-R, Khalil AM, Younes HA, Nehela Y, et al. Chitosan nanoparticles inactivate alfalfa mosaic virus replication and boost innate immunity in *Nicotiana glutinosa* plants. *Plants*. 2021;**10**(12):2701. DOI: 10.3390/plants10122701

[43] El-Ansary MSM, Khalifa EZ, Hemdan SM. Influence of fungal Chitosan to control root-knot nematode *Meloidogyne incognita* on Banana plants. *The Egyptian Journal of Phytopathology*. 2013;**41**(1):43-52. DOI: 10.21608/ejp.2013.101967

[44] Escudero N, Lopez-Moya F, Ghahremani Z, Zavala-Gonzalez EA, Alaguero-Cordovilla A, Ros-Ibañez C, et al. Chitosan increases tomato root colonization by *Pochonia chlamydosporia* and their combination reduces root-knot nematode damage. *Frontiers in Plant Science*. 2017;**8**:1415. DOI: 10.3389/fpls.2017.01415

[45] Khan A, Tariq M, Ahmad F, Mennan S, Khan F, Asif M, et al. Assessment of nematicidal efficacy of chitosan in combination with botanicals against *Meloidogyne incognita* on carrot. *Acta Agriculturae Scandinavica Section B*. 2021;**71**(4):225-236. DOI: 10.1080/09064710.2021.1880620

[46] Singh RR, Chinnasri B, De Smet L, Haeck A, Demeestere K, Van Cutsem P,

et al. Systemic defense activation by COS-OGA in rice against root-knot nematodes depends on stimulation of the phenylpropanoid pathway. *Plant Physiology and Biochemistry*. 2019;**1**(142):202-210. DOI: 10.1016/j.plaphy.2019.07.003

[47] Bistgani ZE, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. *The Crop Journal*. 2017;**5**(5):407-415. DOI: 10.1016/j.cj.2017.04.003

[48] Jiao Z, Li Y, Li J, Xu X, Li H, Lu D, et al. Effects of exogenous chitosan on physiological characteristics of potato seedlings under drought stress and rehydration. *Potato Research*. 2012;**55**(3):293-301. DOI: 10.1007/s11540-012-9223-8

[49] Yang F, Hu J, Li J, Wu X, Qian Y. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. *Plant Growth Regulation*. 2009;**58**(2):131-136. DOI: 10.1007/s10725-009-9361-4

[50] Farouk S, Amany AR. Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian Journal of Biology*. 2012;**2**(10):1341-1358. DOI: 10.21608/JPP.2011.85662

[51] Pirbalouti AG, Malekpoor F, Salimi A, Golparvar A. Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) under reduced irrigation. *Scientia Horticulturae*. 2017;**217**:114-122. DOI: 10.1016/j.scienta.2017.01.031

- [52] Dzung NA, Khanh VTP, Dzung TT. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohydrate Polymers*. 2011;**84**:751-755. DOI: 10.1016/J.CARBPOL.2010.07.066Corpus ID: 85289773
- [53] Bittelli M, Flury M, Campbell GS, Nichols EJ. Reduction of transpiration through foliar application of chitosan. *Agricultural and Forest Meteorology*. 2001;**107**:167-175. DOI: 10.1016/S0168-1923(00)00242-2
- [54] Jabeen N, Ahmad R. The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan. *Journal of the Science of Food and Agriculture*. 2013;**93**(7):1699-1705. DOI: 10.1002/jsfa.5953
- [55] Ma L, Li Y, Yu C, Wang Y, Li X, Li N, et al. Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. *Protoplasma*. 2012;**249**(2):393-399. DOI: 10.1007/s00709-011-0290-5
- [56] Al-Tawaha AR, Turk MA, Al-Tawaha AR, Alu'datt MH, Wedyan M, Al-Ramamneh EA, Hoang AT. Using chitosan to improve growth of maize cultivars under salinity conditions. *Bulgarian Journal of Agricultural Science*. 2018;**24**(3):437-442
- [57] González LM, Guerrero YR, Rodríguez A, Vázquez MN. Effect of seed treatment with chitosan on the growth of rice (*Oryza sativa* L.) seedlings cv. INCA LP-5 in saline medium. *Cultivos Tropicales*. 2015;**36**(1):143-150
- [58] Yahyaabadi HM, Asgharipour MR, Basiri M. Role of chitosan in improving salinity resistance through some morphological and physiological characteristics in fenugreek (*Trigonella foenum-graecum* L.). *Journal of Science and Technology of Greenhouse Culture*. 2016;**7**(25):165-174. DOI: 10.18869/acadpub.ejgcs.7.1.165
- [59] Ibrahim EA, Ramadan WA. Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. *Scientia Horticulturae*. 2015;**184**:101-105. DOI: 10.1016/j.scienta.2014.11.010
- [60] Xue GX, Gao HY, Li PM, Zou Q. Effects of chitosan treatment on physiological and biochemical characteristics in cucumber seedlings under low temperature. *Zhi wu Sheng li yu fen zi Ssheng wu xue xue bao= Journal of Plant Physiology and Molecular Biology*. 2004;**30**(4):441-448
- [61] Guan Y, Hu J, Wang X, Shao C. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of Zhejiang University. Science. B*. 2009;**10**:427-433. DOI: 10.1631/jzus.B0820373
- [62] Sheikha SA, Al-Malki FM. Growth and chlorophyll responses of bean plants to the chitosan applications. *European Journal of Scientific Research*. 2011;**50**(1):124-134
- [63] Chibu H, Shibayama H, Arima S. Effects of chitosan application on the shoot growth of rice and soybean. *Japanese Journal of Crop Science*. 2002;**71**(2):206-211. DOI: 10.1626/jcs.71.206
- [64] Shehata SA, Fawzy ZF, El-Ramady HR. Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Australian Journal of Applied Science*. 2012;**6**(4):63-71

- [65] Mondal MMA, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M, Naher L. Effect of foliar application of chitosan on growth and yield in okra. *Australian Journal of Crop Science*. 2012;**6**(5):918-921
- [66] Sultana S, Islam M, Khatun MA, Hassain M, Huque R. Effect of foliar application of oligo-chitosan on growth, yield and quality of tomato and eggplant. *Asian Journal of Advances in Agricultural Research*. 2017;**11**(2):36-42. DOI: 10.3923/ajar.2017.36.42
- [67] Salachna P, Byczyńska A, Jeziorska I, Udyecz E. Plant growth of *Verbena bonariensis* L. after chitosan, gellan gum or iota-carrageenan foliar applications. *World Science News*. 2017;**62**:111-123
- [68] Ohta K, Atarashi H, Shimatani Y, Matsumoto S, Asao T, Hosoki T. Effects of chitosan with or without nitrogen treatments on seedling growth in *Eustoma grandiflorum* (Raf.) Shinn. cv. Kairyoku Wakamurasaki. *Journal of the Japanese Society for Horticultural Science*. 2000;**69**(1):63-65. DOI: 10.2503/jjshs.69.63
- [69] Khan W, Prithiviraj B, Smith DL. Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. *Journal of Plant Physiology*. 2003;**160**(8):859-863. DOI: 10.1078/0176-1617-00905
- [70] Narula K, Elagamey E, Abdellatef MAE, Sinha A, Ghosh S, Chakraborty N, et al. Chitosan-triggered immunity to *Fusarium* in chickpea is associated with changes in the plant extracellular matrix architecture, stomatal closure and remodelling of the plant metabolome and proteome. *The Plant Journal*. 2020;**103**(2):561-583. DOI: 10.1111/tpj.14750
- [71] Iriti M, Faoro F. Chitosan as a MAMP, searching for a PRR. *Plant Signaling & Behavior*. 2009;**4**(1):66-68. DOI: 10.4161/psb.4.1.7408
- [72] Köhle H, Jeblick W, Poten F, Blaschek W, Kauss H. Chitosan-elicited callose synthesis in soybean cells as a Ca²⁺-dependent process. *Plant Physiology*. 1985;**77**(3):544-551. DOI: 10.1104/pp.77.3.544
- [73] Gow NAR, Latge JP, Munro CA. The fungal cell wall: Structure, biosynthesis, and function. *Microbiology Spectrum*. 2017;**5**(3):5-3. DOI: 10.1128/microbiolspec.FUNK-0035-2016
- [74] Benhamou N. Ultrastructural and cytochemical aspects of chitosan on *fusarium oxysporum* f. sp. *radicis-lycopersici*, agent of tomato crown and root rot. *Phytopathology*. 1992;**82**:1185-1193. DOI: 10.1016/S0885-5765(05)80098-0
- [75] Ait Barka E, Eullaffroy P, Clément C, Vernet G. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Reports*. 2004;**22**:608-614. DOI: 10.1007/s00299-003-0733-3
- [76] Baños SB, López MH, Molina EB. Growth inhibition of selected fungi by chitosan and plant extracts. *Revista Mexicana de Fitopatología*. 2004;**22**(2):178-186
- [77] Pasquina-Lemonche L, Burns J, Turner RD, Kumar S, Tank R, Mullin N, et al. The architecture of the gram-positive bacterial cell wall. *Nature*. 2020;**582**(7811):294-297. DOI: 10.1038/s41586-020-2236-6
- [78] Cheung RC, Ng TB, Wong JH, Chan WY. Chitosan: An update on potential biomedical and pharmaceutical applications. *Marine Drugs*. 2015;**13**:5156-5186. DOI: 10.3390/md13085156
- [79] Chung YC, Su YP, Chen CC, Jia G, Wang HL, Wu JG, et al. Relationship

between antibacterial activity of chitosan and surface characteristics of cell wall. *Acta Pharmacologica Sinica*. 2004;**25**(7):932-936

[80] Tan H, Ma R, Lin C, Liu Z, Tang T. Quaternized chitosan as an antimicrobial agent: Antimicrobial activity, mechanism of action and biomedical applications in orthopedics. *International Journal of Molecular Sciences*. 2013;**14**(1):1854-1869. DOI: 10.3390/ijms14011854

[81] Farag SMA, Elhalag KMA, Mohamed H, Hagag MH, Khairy ASM, Ibrahim HM, et al. Potato bacterial wilt suppression and plant health improvement after application of different antioxidants. *Journal of Phytopathology*. 2017;**65**:522-537. DOI: 10.1111/jph.12589

[82] Li J, Wu Y, Zhao L. Antibacterial activity and mechanism of chitosan with ultra-high molecular weight. *Carbohydrate Polymers*. 2016;**148**:200-205. DOI: 10.1016/j.carbpol.2016.04.025

[83] Annaian S, Kandasamy K, Lakshman N. Preparation, characterization and antibacterial activity of chitosan and phosphorylated chitosan from cuttlebone of *Sepia kobeensis*. *Biotechnology Reports*. 2016;**9**:25-30. DOI: 10.1016/j.btre.2015.10.007

[84] Sapers GM. Chitosan enhances control of enzymatic browning in apple and pear juice by filtration. *Journal of Food Science*. 1992;**57**(5):1192-1193. DOI: 10.1111/j.1365-2621.1992.tb11296.x

[85] Chung YC, Wang HL, Chen YM, Li SL. Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. *Bioresource Technology*. 2003;**88**:179-184. DOI: 10.1016/s0960-8524(03)00002-6

[86] Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as

antimicrobial agent: Applications and mode of action. *Biomacromolecules*. 2003;**4**(6):1457-1465. DOI: 10.1021/bm034130m

[87] Goy RC, Morais ST, Assis OB. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth. *Revista Brasileira de Farmacognosia*. 2016;**26**:122-127. DOI: 10.1016/j.bjp.2015.09.010

[88] Liang C, Yuan F, Liu F, Wang Y, Gao Y. Structure and antimicrobial mechanism of ϵ -polylysine-chitosan conjugates through Maillard reaction. *International Journal of Biological Macromolecules*. 2014;**70**:427-434. DOI: 10.1016/j.ijbiomac.2014.07.012

[89] Acar O, Aki C, Erdugan H. Fungal and bacterial diseases control with Elexa TM plant booster. *Fresenius Environmental Bulletin*. 2008;**17**:797-802

[90] Li B, Wang X, Chen RX, Huangfu WG, Xie GL. Antibacterial activity of chitosan solution against *Xanthomonas* pathogenic bacteria isolated from *Euphorbia pulcherrima*. *Carbohydrate Polymers*. 2008;**72**:287-292. DOI: 10.1016/j.carbpol.2007.08.012

[91] Dong OX, Ronald PC. Genetic engineering for disease resistance in plants: Recent progress and future perspectives. *Plant Physiology*. 2019;**180**:26-38. DOI: 10.1104/pp.18.01224

[92] Mansouri S, Lavigne P, Corsi K, Benderdour M, Beaumont E, Fernandes JC. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy strategies to improve transfection efficacy. *European Journal of Pharmaceutics and Biopharmaceutics*. 2004;**57**:1-8. DOI: 10.1016/s0939-6411(03)00155-3

[93] Akhter G, Khan TA. Evaluation of some plant extracts for nemato-toxic

- potential against juveniles of *Meloidogyne incognita* *in vitro*. *The Journal of Phytopharmacology*. 2018;7(2):141-145. DOI: 10.31254/phyto.2018.7207
- [94] Pichyangkura R, Chadchawan S. Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae*. 2015;196:49-65. DOI: 10.1016/j.scienta.2015.09.031
- [95] Din J, Khan SU, Ali I, Gurmani AR. Physiological and agronomic response of canola varieties to drought stress. *The Journal of Animal and Plant Sciences*. 2011;21(1):78-82. DOI: 10.4081/ijas.2011.e11
- [96] Li Q, Zhang X, Lv Q, Zhu D, Qiu T, Xu Y, et al. Physcomitrella Patens dehydrins (PpDHNA and PpDHNC) confer salinity and drought tolerance to transgenic Arabidopsis plants. *Frontiers in Plant Science*. 2017;8:1316. DOI: 10.3389/fpls.2017.01316
- [97] Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. Application of chitosan on plant responses with special reference to abiotic stress. *Physiology and Molecular Biology of Plants*. 2019;25(2):313-326. DOI: 10.1007/s12298-018-0633-1
- [98] Nazarli H, Faraji F, Zardashti MR. Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. *Cercetari Agronomice in Moldova*. 2011;1(145):35-41
- [99] Lai QX, Bao ZY, Zhu ZJ, Qian QQ, Mao BZ. Effects of osmotic stress on antioxidant enzymes activities in leaf discs of PSAG12-IPT modified *Gerbera*. *Journal of Zhejiang University. Science B*. 2007;8(7):458-464. DOI: 10.1631/jzus.2007.B0458
- [100] Khan WM, Prithiviraj B, Smiyh DL. Effect of foliar application of chitinoligosaccharides on photosynthesis of maize and soybean. *Photosynthetica*. 2002;40(4):621-624. DOI: 10.1023/A:1024320606812
- [101] Meloni DA, Oliva MA, Martinez CA, Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*. 2003;49(1):69-76. DOI: 10.1016/S0098-8472(02)00058-8
- [102] Sun Z, Shi C, Wang X, Fang Q, Huang J. Synthesis, characterization, and antimicrobial activities of sulfonated chitosan. *Carbohydrate Polymers*. 2017;2(155):321-328
- [103] Khan MN, Siddiqui MH, Mohammad F, Naeem M, Khan MMA. Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defence system and osmoprotectant accumulation. *Acta Physiologiae Plantarum*. 2010;32:121-132. DOI: 10.1007/s11738-009-0387-z
- [104] Choi YS, Kim YM, Hwang OJ, Han YJ, Kim SY, Kim JI. Overexpression of Arabidopsis ABF3 gene confers enhanced controlled-release and water-retention. *Carbohydrate Polymers*. 2008;72:240-247. DOI: 10.1007/s11816-012-0245-0
- [105] Torres-Rodriguez JA, Reyes-Perez JJ, Castellanos T, Angulo C, Quinones-Aguilar EE, Hernandez-Montiel LG. A biopolymer with antimicrobial properties and plant resistance inducer against phytopathogens: Chitosan. *Not Bot Horti Agrobo*. 2021;49(1):12231. DOI: 10.15835/nbha49112231
- [106] Hameed A, Sheikh M, Hameed A, Farooq T, Basra S, Jamil A. Chitosan priming enhances the seed germination, antioxidants, hydrolytic enzymes,

soluble proteins and sugars in wheat seeds. *Agrochimica*. 2013;**67**:32-46

[107] Ziani K, Ursúa B, Maté JI. Application of bioactive coatings based on chitosan for artichoke seed protection. *Crop Protection*. 2010;**29**(8):853-859. DOI: 10.1016/j.cropro.2010.03.002

[108] Saavedra GM, Figueroa NE, Poblete LA, Cherian S, Figueroa CR. Effects of preharvest applications of methyl jasmonate and chitosan on postharvest decay, quality and chemical attributes of *Fragaria chiloensis* fruit. *Food Chemistry*. 2016;**1**(190):448-453. DOI: 10.1016/j.foodchem.2015.05.107

[109] Kowalski B, Terry FJ, Herrera L, Peñalver DA. Application of soluble chitosan *in vitro* and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Research*. 2006;**49**:167-176. DOI: 10.1007/s11540-006-9015-0

[110] González Gómez H, Ramírez Godina F, Ortega Ortiz H, Benavides Mendoza A, Robledo TV, Cabrera De la Fuente M. Use of chitosan-PVA hydrogels with copper nanoparticles to improve the growth of grafted watermelon. *Molecules*. 2017;**22**:1031. DOI: 10.3390/molecules22071031

[111] Amin AA, Rashad EL-SM, EL-Abagy HMH. Physiological effect of indole-3-butyric acid and salicylic acid on growth, yield and chemical constituents of onion plants. *Journal of Applied Sciences Research*. 2007;**3**(11):1554-1563

[112] Dzung NA, Thang NTE. Effect of oligoglucosamine prepared by enzyme degradation on the growth of soybean. *Advances in Chitin and Chitosan Science*. 2002;**5**:463-467

[113] Rahman M, Mukta JA, Sabir AA, Gupta DR, Mohi-Ud-Din M,

Hasanuzzaman M, et al. Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. *PLoS One*. 2018;**13**(3):e0203769. DOI: 10.1371/journal.pone.0203769

[114] Hadwiger LA. Multiple effects of chitosan on plant systems: Solid science or hype. *Plant Science*. 2013;**208**:42-49. DOI: 10.1016/j.plantsci.2013.03.007

[115] Lin W, Hu X, Zhang W, Rogers WJ, Cai W. Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice. *Journal of Plant Physiology*. 2005;**162**:937-944. DOI: 10.1016/j.jplph.2004.10.003

[116] Hoat TX, Nakayashiki H, Yang Q, Tosa Y, Mayama S. Molecular cloning of the apoptosis-related calcium-binding protein AsALG-2in *Avena sativa*. *Molecular Plant Pathology*. 2013;**14**(3):222-229. DOI: 10.1111/j.1364-3703.2012.00844.x

[117] Ma ZX, Yang LY, Yan HX, Kennedy JF, Meng XH. Chitosan and oligochitosan enhance the resistance of peach fruit to brown rot. *Carbohydrate Polymers*. 2013;**94**(1):272-277. DOI: 10.1016/j.carbpol.2013.01.012

[118] Wang SY, Gao H. Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria x ananassa* Duch.). *LWT-Food Science and Technology*. 2013;**52**(2):71-79. DOI: 10.1016/j.lwt.2012.05.003

[119] Siddaiah CN, Prasanth KV, Satyanarayana NR, Mudili V, Gupta VK, Kalagatur NK, et al. Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. *Scientific*

Reports. 2018;**8**(1):1-4. DOI: 10.1038/s41598-017-19016-z

[120] Borsani O, Valpuesta V, Botella MA. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology*. 2001;**126**(3):1024-1030. DOI: 10.1104/pp.126.3.1024

[121] Karthikeyan M, Jayakumar V, Radhika K, Bhaskaran R, Velazhahan R, Alice D. Induction of resistance in host against the infection of leaf blight pathogen (*Alternaria palandui*) in onion (*Allium cepa* var *aggregatum*). *Indian Journal of Biochemistry & Biophysics*. 2005;**42**(6):371-377

[122] Yang TP, Poovaiah BW. Hydrogen peroxide homeostasis: Activation of plant catalase by calcium/calmodulin. *The Proceedings of the National Academy of Sciences*. 2002;**99**(6):4097-4102. DOI: 10.1073/pnas.052564899

[123] Xu QJ, Nian YG, Jin XC, Yan CZ, Jin LI, Jiang GM. Effects of chitosan on growth of an aquatic plant (*Hydrilla verticillata*) in polluted waters with different chemical oxygen demands. *Journal of Environmental Sciences*. 2007;**19**(2):217-221. DOI: 10.1016/S1001-0742(07)60035-7

[124] MacDonald MJ, D'Cunha GB. A modern view of phenylalanine ammonia lyase. *Biochemistry and Cell Biology*. 2007;**85**:273-282. DOI: 10.1139/o07-018

[125] Xing K, Zhu X, Peng X, Qin S. Chitosan antimicrobial and eliciting properties for pest control in agriculture: A review. *Agronomy for Sustainable Development*. 2015;**35**(2):569-588. DOI: 10.1007/s13593-014-0252-3

[126] Luna E, Pastor V, Robert J, Flors V, Mauch-Mani B, Ton J. Callose deposition: A multifaceted plant defense response.

Molecular Plant-Microbe Interactions. 2011;**24**(2):183-193. DOI: 10.1094/mpmi-07-10-0149

[127] Mejía-Teniente L, de Dalia Durán-Flores F, Chapa-Oliver AM, Torres-Pacheco I, Cruz-Hernández A, González-Chavira MM, et al. Oxidative and molecular responses in *Capsicum annuum* L. after hydrogen peroxide, salicylic acid and chitosan foliar applications. *International Journal of Molecular Sciences*. 2013;**14**(5):10178-10196. DOI: 10.3390/ijms140510178

[128] Blume B, Nürnberger T, Nass N, Scheel D. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. *The Plant Cell*. 2000;**12**(8):1425-1440. DOI: 10.1105/tpc.12.8.1425

[129] Klüsener B, Young JJ, Murata Y, Allen GJ, Mori IC, Hugouvieux V, et al. Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in *Arabidopsis* guard cells. *Plant Physiology*. 2002;**130**(4):2152-2163. DOI: 10.1104/pp.012187

[130] Tocci N, Simonetti G, D'Auria FD, Panella S, Palamara AT, Valletta A, et al. Root cultures of *Hypericum perforatum* subsp. *angustifolium* elicited with chitosan and production of xanthone-rich extracts with antifungal activity. *Applied Microbiology and Biotechnology*. 2011;**91**:977-987. DOI: 10.1007/s00253-011-3303-6

[131] Halim VA, Vess A, Scheel D, Rosahl S. The role of salicylic acid and jasmonic acid in pathogen defence. *Plant Biology*. 2006;**8**(03):307-313. DOI: 10.1055/s-2006-924025

[132] Rakwal R, Tamogami S, Agrawal GK, Iwahashi H. Octadecanoid signaling component "burst" in rice (*Oryza sativa* L.) seedling leaves upon

wounding by cut and treatment with fungal elicitor chitosan. *Biochemical and Biophysical Research Communications*. 2002;**295**:1041-1045. DOI: 10.1016/S0006-291X(02)00779-9

[133] Seo M, Koshiha T. Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science*. 2002;**7**:41-48. DOI: 10.1016/S1360-1385(01) 02187-2

[134] Gutjahr C, Parniske M, Schekman R. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology*. 2013;**29**:593-617. DOI: 10.1146/annurev-cellbio-101512-122413

[135] Li K, Xing R, Liu S, Li P. Chitin and chitosan fragments responsible for plant elicitor and growth stimulator. *Journal of Agricultural and Food Chemistry*. 2020;**68**(44):12203-12211

[136] Liang Y, Tóth K, Cao Y, Tanaka K, Espinoza C, Stacey G. Lipochitoooligosaccharide recognition: An ancient story. *The New Phytologist*. 2014;**204**:289-296. DOI: 10.1111/nph.12898

[137] O'Herlihy EA, Duffy EM, Cassells AC. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folia Geobotanica*. 2003;**38**:201-207. DOI: 10.1007/BF02803152

[138] Savci S. Investigation of effect of chemical fertilizers on environment. *APCBEE Procedia*. 2012;**1**(1):287-292. DOI: 10.1016/j.apcbee.2012.03.047

[139] Hussain MR, Devi RR, Maji TK. Controlled release of urea from chitosan microspheres prepared by emulsification and cross-linking method. *Iranian Polymer Journal*. 2012;**21**:473-479. DOI: 10.1007/s13726-012-0051-0

[140] Quesada HB, Baptista ATA, Cusioli LF, Seibert D, Bezerra CO, Bergamasco R. Surface water pollution by pharmaceuticals and an alternative of removal by low-cost adsorbents: A review. *Chemosphere*. 2019;**222**:766-780. DOI: 10.1016/j.chemosphere.2019.02.009

[141] Zhao L, Guan X, Yu B, Ding N, Liu X, Ma Q, et al. Carboxylated graphene oxide-chitosan spheres immobilize Cu²⁺ in soil and reduce its bioaccumulation in wheat plants. *Environment International*. 2019;**133**:105208. DOI: 10.1016/j.envint.2019.105208

[142] Qian J, Zhou L, Yang X, Hua D, Wu N. Prussian blue analogue functionalized magnetic microgels with ionized chitosan for the cleaning of cesium-contaminated clay. *Journal of Hazardous Materials*. 2020;**386**:121965. DOI: 10.1016/j.jhazmat.2019.121965

[143] Türker OC, Baran TA. Combination method based on chitosan adsorption and duckweed (*Lemna gibba* L.) phytoremediation for boron (B) removal from drinking water. *International Journal of Phytoremediation*. 2018;**20**(2):175-183. DOI: 10.1080/15226514.2017.1350137

[144] Sabeen AH, Kamaruddin SN, Noor ZZ. Environmental impacts assessment of industrial wastewater treatment system using electroless nickel plating and life cycle assessment approaches. *International Journal of Environmental Science and Technology*. 2019;**16**(7):3171-3182. DOI: 10.1007/s13762-018-1974-6

[145] Pérez Mora BE, Bellú SE, Mangiameli MF, García SI, González JC. Optimization of continuous arsenic biosorption present in natural contaminated groundwater. *Journal of Chemical Technology & Biotechnology*. 2019;**94**(2):547-555. DOI: 10.1002/jctb.5801

- [146] Taha AA, Hameed NJ, Rashid FH. Preparation and characterization of (hyacinth plant/chitosan) composite as a heavy metal removal. *Baghdad Science Journal*. 2019;**16**(4):865-870. DOI: 10.21123/bsj.2019.16.4.0865
- [147] Xiang J, Lin Q, Yao X, Yin G. Removal of Cd from aqueous solution by chitosan coated MgO-biochar and its in-situ remediation of Cd-contaminated soil. *Environmental Research*. 2021;**1**(195):110650. DOI: 10.1016/j.envres.2020.110650
- [148] Dinh VP, Nguyen MD, Nguyen QH, Do TTT, Luu TT, Luu AT, et al. Chitosan-MnO₂ nanocomposite for effective removal of Cr (VI) from aqueous solution. *Chemosphere*. 2020;**257**:127-147. DOI: 10.1016/j.chemosphere.2020.127147
- [149] Hu D, Lian Z, Xian H, Jiang R, Wang N, Weng Y, et al. Adsorption of Pb (II) from aqueous solution by polyacrylic acid grafted magnetic chitosan nanocomposite. *International Journal of Biological Macromolecules*. 2020;**154**:1537-1547. DOI: 10.1016/j.ijbiomac.2019.11.038 Epub November 13, 2019
- [150] Lobo C, Castellari J, Lerner JC, Bertola N, Zaritzky N. Functional iron chitosan microspheres synthesized by ionotropic gelation for the removal of arsenic (V) from water. *International Journal of Biological Macromolecules*. 2020;**164**:1575-1583. DOI: 10.1016/j.ijbiomac.2020.07.253
- [151] Zong H, Li K, Liu S, Song L, Xing R, Chen X, et al. Improvement in cadmium tolerance of edible rape (*Brassica rapa* L.) with exogenous application of chitooligosaccharide. *Chemosphere*. 2017;**1**(181):92-100. DOI: 10.1016/j.chemosphere.2017.04.024
- [152] Rychter P. Chitosan/glyphosate formulation as a potential, environmental friendly herbicide with prolonged activity. *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes*. 2019;**54**(8):681-692. DOI: 10.1080/03601234.2019.1632644
- [153] Rashidipour M, Maleki A, Kordi S, Birjandi M, Pajouhi N, Mohammadi E, et al. Pectin/chitosan/tripolyphosphate nanoparticles: Efficient carriers for reducing soil sorption, cytotoxicity, and mutagenicity of paraquat and enhancing its herbicide activity. *Journal of Agricultural and Food Chemistry*. 2019;**67**:5736-5745. DOI: 10.1021/acs.jafc.9b01106
- [154] Yuan X, Wu Z, Li H, Wang Y, Liu F, Cai H, et al. Biochemical and proteomic analysis of 'Kyoho' grape (*Vitis labruscana*) berries during cold storage. *Postharvest Biology and Technology*. 2014;**88**:79-87. DOI: 10.1016/j.postharvbio.2013.10.001
- [155] Li M, Chen C, Xia X, Garba B, Shang L, Wang Y. Proteomic analysis of the inhibitory effect of chitosan on *Penicillium expansum*. *Food Science & Technology*. 2019;**40**:250-257. DOI: 10.1590/fst.40418