




Review

# Mechanism of Plant Growth Promotion and Disease Suppression by Chitosan Biopolymer

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**Abstract:** The chitosan (CHT) biopolymer is a de-acetylated chitin derivative that exists in the outer shell of shrimp, shellfish, lobster or crabs, as well as fungal cell walls. Because of its biodegradability, environmental non-toxicity, and biocompatibility, it is an ideal resource for sustainable agriculture. The CHT emerged as a promising agent used as a plant growth promoter and also as an antimicrobial agent. It induces plant growth by influencing plant physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation and synthesis of protein that can eventually lead to increased yield. It also acts as a catalyst to inhibit the growth of plant pathogens, and alter plant defense responses by triggering multiple useful metabolic pathways. This review emphasizes the role and mechanisms of CHT as a plant growth promoter and disease suppressor, and its future implications in agriculture.

**Keywords:** antimicrobial agent; biopolymer; chitosan; defense mechanism; growth promoter; structural diversity

## 1. Introduction

Chitosan (CHT) is a poly (1,4)-2-amino-2-deoxy- $\beta$ -D glucose, a de-acetylation derivative of chitin, found in arthropod exoskeletons, which includes crustaceans like lobsters, shrimps and crabs, insects, mollusc radulae, beaks of cephalopod and fish, and lissamphibian scales [1]. The discovery of Chitosan (pronounced as *Kite-O-San*) dates back to 1811, when a French Professor Henri Braconnot of Natural History first found “chitin” from which it is derived. He found a mushroom extract which would not dissolve in sulphuric acid, and he called it ‘fungine’ [2,3]. In 1823, it was named ‘chitin’ after another scientist Auguste Odier extracted it from cuticles of beetle and called it ‘chiton’. Chitin was the first man-identified polysaccharide, about 30 years prior to cellulose. The concept was further recognized when the existence of nitrogen in the chitin was demonstrated by Lassaigne in 1843. Professor C. Rouget undertook the alkaline treatment of chitin in 1859, resulting in an acid dissoluble substance,

unlike chitin itself. Hoppe-Seiler gave the name “chitosan” to de-acetylated chitin [4]. Although chitin has long been an unused natural component, interest in this biopolymer and its derivatives like CHT has grown significantly in recent years due to its diversified biological properties.

The biopolymer CHT is safe, cheap and its chemical structure can easily be converted to develop relevant polymers for specified applications. These features make CHT a molecule of great significance in a wide range of potential users, from health care and biotechnological industries to farmers [5,6]. It is biodegradable, environment friendly for agriculture, and not toxic to humans or other organisms [7]. It has shown efficacy in reducing disease incidence and increasing crop growth, yield, and quality. The CHT has been documented as an elicitor of plants' natural defense response, and has been utilized as a natural product to combat pathogenic diseases before and after harvest [8]. It functions as an antifungal [9], antibacterial [10], antiviral [11], and bionematicidal agent [12]. Chitosan has been widely utilized as a coating agent of different nuts, cereals, fruits, and vegetables to protect from post-harvest losses, and increase the duration of storage and preservation [13,14]. A wide range of studies showed that foliar application of CHT improves plant growth, yield and induces synthesis of secondary metabolites like polyphenolics, flavonoids, lignin, and phytoalexins in plants [15,16]. It influences seed plasma membrane permeability, enhances sugar and proline concentration, boosts peroxidase (POD), phenylalanine ammonia-lyase (PAL), tyrosine ammonialyase (TAL) and catalase (CAT) activities [17]. This article will focus on the effects and mechanisms of CHT as a plant growth promoter and disease suppressor, as well as its future implications in agriculture.

## 2. Chitosan and Its Structural Diversity

Chitosan is a linear biopolymer comprising two sub-units, i.e., D-glucosamine and N-acetyl-D-glucosamine, connected by 1,4-glycosidic bonds to each other [18,19]. There are three rings in the structure of the CHT molecule. CHT displays three functional groups, primary and secondary groups of hydroxyls and amine. CHT also contains beta-1, 4 glycosidic bonds. The oxygen atoms (O1 and O2) are bound to the atoms of C6–C7 and C10–C13 [20]. These functional groups allow them to undergo chemical modifications easily. Chemical derivatives of CHT have gained increasing emphasis over the last decade owing to their biological, chemical and functional benefits over unmodified CHT in terms of solubility, gelling properties, nature of amphiphilic hydrophobic variants, and ability to manipulate chemical conjugates and self-assembling nanostructures, and enhanced biocompatibility [21]. Modification can be achieved through physical or chemical techniques such as cross-linking, grafting, incorporation of substituents or composites. Chitosan possesses many reactive amino side groups that improve CHT's applicability and provide the possibility of developing a broad range of CHT derivatives.

Oligochitosan is one of the important water-soluble CHT derivatives. Along with other polysaccharides, CHT can also be hydrolyzed by biodegrading agents owing to its unstable glycosidic linkages. Oligochitosan can be developed by various methods like hydrolysis of acids [22], oxidative degradation [23], enzymatic hydrolysis [24], and ultrasonic degradation [25]. The amphiphilic properties of CHT derivatives have significantly enhanced their solubility and capacity to be self-assembled by intra- and intermolecular interaction of hydrophobic moieties as aggregates and micelles. It acts as an outstanding model for drug delivery and improves gene therapy transfection [26]. Hydroxyalkyl CHTs are formed when CHT reacts with epoxide. Self-assembled glycol CHT-based nanoparticles were prepared as a drug carrier [27]. Another cationic water-soluble derivative of CHT is the trimethylchitosan ammonium. It is formed due to the quaternization of CHT, i.e., by reacting with methyl iodide and sodium hydroxide to lower CHT acetyl content. Trimethylchitosan ammonium exhibits flocculating qualities such as dispersions of kaolin, making it essential in paper processing [28]. CHT's mucoadhesive characteristics have been enhanced through the thiol group immobilization on polymer. Thiolated CHT improves permeation and shows excellent coherent properties for extended, regulated delivery of embedded therapeutic substances [29].

N-carboxymethyl CHT is a water-soluble CHT derivative with a broad range of uses in the food, medical, and gene therapy sectors [30]. It can be produced by glyoxylic acid treatment of CHT. It is required to build various protein drug delivery systems, like super porous hydrogels, cross-linked hydrogels, and pH-sensitive hydrogels [31]. N-methylene phosphonic CHT (NMPC) is an anionic derivative that exhibits amphoteric characteristics. NMPC has cation-binding efficacy, like  $\text{Ca}^{2+}$  and several transition metals ( $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ) [32]. N-arylated CHT has a hydrophobic nature as well as hydrophilic and nucleophilic properties at the atom of nitrogen [33].

CHT sulfates, besides their antisclerotic, antibacterial, antiviral, antioxidant, and enzyme inhibition functions, have been shown to have anticoagulant and heamagglutination action owing to a structural resemblance to heparin. They also have excellent adsorption capabilities and are used for the recovery of metal ions. Compounds of sulfur are grafted onto CHT for mercury recovery and precious metal uptake. Sulphonic CHTs are fine metallic oxide flocculants [34]. Another functional by-product of CHT, lactic-glycolic acid-chitosan hydrogels, show greater interactions between water and CHT chains. These may be generated without any catalyst by direct grafting of D, L-lactic, or glycolic acid on CHT. This has significant usage for drug delivery systems and wound dressings in the biomedical sector [35]. CHT bonded with sugar has unique receptor-binding characteristics and is being studied for its antiviral impacts [36]. A CHT-containing cyclodextrin pendant has been produced to improve the drug delivery systems, cosmetics, and textile effluent decontamination [37].

Modifications of CHT with phosphorylcholine substances offer anticoagulant properties. Enzymatic grafting of phenolic substances onto CHT has been documented utilizing tyrosinase to impart water solubility under specific conditions [38]. DNA delivery is performed using grafted CHTs such as PEG-CHT, galactosylated CHT, etc. [39]. Most of the hydrogels grafted with polyacryl show stimulus-sensitive behavior such as pH or temperature, or both [40]. CHT salts such as formate, lactate, acetate, malate, citrate, glyoxylate, tartarate, pyruvate, malonate, ascorbate, and glycolate are soluble in water. CHT is most desired for its charges and diverse functional groups that make it possible to exploit into several variants with applications in different areas [41].

### 3. Effect of Chitosan Biopolymer on Plant Growth

Chitosan functions as a plant growth promoter in various crops such as beans, potato, radish, gerbera, soybean, cabbage, and other crops. As a result of plant growth promotion, it also enhances yield. Chitosan has a major influence on the growth rates of shoots, roots, flowering, and the number of flowers. As chitosan molecules are extremely hydrophilic, they reduce stress damage in plant cells by decreasing water content and accelerating several biological macromolecules' activities. Three trials were conducted on orchids to determine the effect of CHT on organogenesis; the results showed that CHT could produce positive results at a very low concentration [42–44]. The results also suggested that CHT was working as a consequence of other metabolic processes rather than merely enhancing nitrogen nutritional quality or as a source of energy for the production of carbohydrates. Both Pornpeanpakdee et al. [43] and Nahar et al. [44] found that orchid growth (*Dendrobium* and *Cymbidium*) was stimulated by the supply of CHT to micropropagated plants that grow under sterile conditions. This is corroborated by other findings showing increased growth in aseptic conditions like tissue cultured grapes [45] and the growth of *Phyla dulcis* in liquid bioreactors [46].

Significant growth improvements have been found by several studies in daikon radishes [47], cabbage [48], soybean sprouts [49], sweet basil [50], and also in ornamental crops, including *Gerbera* [51] and *Dendrobium* orchids [42] by various modes of application such as in vitro, in vivo, soil application, pot application and biofertilization. To increase maize yield, a mixture of CHT and plant-growth-promoting rhizobacteria can be utilized as biofertilizers [52]. It is utilized in potted freesia cultivation as a biostimulator [53]. Vasudevan et al. [54] reported that the use of CHT formulation could accelerate the length of root and shoot and yield of rice grain. It also promotes the growth of plants such as pepper, cucumber and tomato raised in the nursery. Therefore, we have enlisted

some important agricultural crops that showed improved plant growth and development due to the application of CHT (Table 1).

**Table 1.** Effects of chitosan (CHT) on plant growth and development.

Plant Species	CHT Effects	Mode of Application	References
Rice ( <i>Oryza sativa</i> L.)	Increased plant growth, higher photosynthesis rate	In vivo	[55]
Soybean ( <i>Glycine max</i> )	Increased plant growth	Soil application	[56]
Rape ( <i>Brassica rapa</i> L.)	Increased plant growth and content of leaf chlorophyll	Hydroponic pot application	[57]
Maize ( <i>Zea mays</i> L.)	Increased plant growth and grain weight	Biofertilization	[52]
	Improved seed germination	In vivo	[58]
	Improved seed germination and vigor index	In vivo	[59]
Potato ( <i>Solanum tuberosum</i> L.)	Increased of tuber size	In vivo	[60]
	Increased plant growth and yield	In vitro and in vivo	[61]
Tomato ( <i>Solanum lycopersicum</i> )	Improved fruit quality and productivity	In vivo	[9,10]
	Increased seed germination and vigor index	In vivo	[62]
Daikon radishes ( <i>Raphanus sativus</i> )	Increased plant growth	In vivo	[47]
Cabbage ( <i>Brassica oleracea</i> )	Increased plant growth	In vivo	[48]
Soybean sprouts ( <i>Glycine max</i> )	Increased plant growth	In vivo	[49]
Okra ( <i>Hibiscus esculentus</i> L.)	Increased plant growth, and yield	In vivo	[63]
Eggplant ( <i>Solanum melongena</i> )	Increased plant growth, and yield	In vivo	[64]
Bean ( <i>Phaseolus vulgaris</i> )	Increased leaf area, and carotenoids and chlorophylls levels	In vitro	[65]
Chili ( <i>Capsicum frutescens</i> L.)	Increased plant growth, yield, and thousand seed weight	In vivo	[66]
	Increased leaf area, canopy diameter, and plant height	In vivo	[67]
Bell pepper ( <i>Capsicum annuum</i> )	Increased fruit weight, diameter, and yield	In vivo	[68]
Turmeric ( <i>Curcuma longa</i> )	Increased plant growth, and yield	In vivo	[69]
Ajowan ( <i>Carum copticum</i> )	Increased seed germination, vigor index, dry weight and radical length	In vivo	[70]
Artichoke ( <i>Cynara scolymus</i> )	Improved seed germination and plant growth	In vivo	[71]
Cucumber ( <i>Cucumis sativus</i> )	Increased plant growth and improved quality	In vivo	[72]

Table 1. Cont.

Plant Species	CHT Effects	Mode of Application	References
Chickpea ( <i>Cicer arietinum</i> )	Increased plant growth	In vivo	[73]
	Increased seed germination and vigor index	In vivo	[74]
Coffee ( <i>Coffea arabica</i> )	Increased plant height and leaf area	In vivo	[75]
Strawberry ( <i>Fragaria × annanasa</i> )	Increased fruit yield and total antioxidant activities	In vivo	[14]
	Increased fruit yield	In vivo	[76]
Watermelon ( <i>Citrullus lanatus</i> )	Increased plant growth	In vivo	[77]
Mango ( <i>Mangifera indica</i> )	Increased plant growth, fruit size and weight	In vivo	[78]
Grapevine ( <i>Vitis vinifera</i> L.)	Increased plant growth	In vivo	[45]
Basil ( <i>Ocimum ciliatum</i> and <i>Ocimum basilicum</i> )	Increased plant growth and phenol content	In vivo	[50]
<i>Phyla dulcis</i>	Increased plant growth	In vitro	[79]
Freesia ( <i>Freesia corymbosa</i> )	Increased plant growth	In vivo	[53]
<i>Gerbera jamesonii</i>	Increased plant growth	In vivo	[51]
<i>Dendrobium aggregatum</i>	Increased plant growth	In vitro	[42]
<i>Cymbidium insigne</i>	Increased plant growth	In vitro	[44]
Kemiri sunan ( <i>Reutealis trisperma</i> )	Increased plant growth	In vivo	[80]
Scots pine ( <i>Pinus sylvestris</i> L.)	Increased plant growth	In vivo	[81]

#### 4. Suppression of Plant Disease by Chitosan

Chitosan has gained popularity in recent years as an environmentally friendly approach to controlling crop diseases [82]. Among the most documented properties of CHT is its effective antimicrobial activity against a wide range of micro-organisms, including fungi, bacteria, viruses, and nematodes. An antimicrobial component is classified as a component that kills micro-organisms or suppresses their growth [83].

##### 4.1. Antifungal Activity of CHT

Since Allan and Hadwiger [84] documented CHT as a bio-fungicide in 1979, it has gained considerable interest in terms of plant protection research. Fungicidal efficacy of CHT against different species of fungi and Oomycetes has been reported [85,86]. It prevents the growth of several pathogenic fungi in vitro, such as *Alternaria alternata*, *Botrytis cinerea*, *Penicillium digitatum*, *Colletotrichum gleosporoides*, *Rhizopus stolonifera*, etc. The suppression was found in different stages of pathogen development, including hyphal growth, spore formation, spore viability, germination, and fungal virulence factor production [87]. El Ghaouth et al. [88] found that CHT has been effective in completely suppressing the mycelial development of *Pythium aphanidermatum*. In the study on *Phytophthora capsici* in peppers by Xu et al. [89], it was observed that the major impact detected in the pathogen was the disturbance of the endomembrane system, particularly the vacuoles' integrity. It controlled damping-off [90], *Alternaria* blight in tomato [10], and inhibited the growth of *Fusarium oxysporum* f. sp.

*tracheiphilum* [91]. CHT successfully prevents the radial hyphal growth, spore formation, germination of spore, and elongation of *Fusarium* spp. [90,92], *Rhizopus* spp. [93,94], *Penicillium* spp. [95], *Phytophthora* spp. [96,97], *Botrytis* spp. [98] and *Alternaria* spp. [10]. The major advantage of CHT use is that it can be formulated and utilized to improve its antifungal activity as a natural antifungal agent in nanoparticles and many other forms. Ing et al. [99] documented an improved inhibitory effect of CHT nanoparticles against *Candida albicans* and *Fusarium solani* compared with the regular form of CHT. Chitosan silver nanoparticles suppressed *Colletotrichum gloeosporioides* conidia germination more efficiently than CHT alone [100].

CHT also has potential in controlling fungal diseases like root rot (*Bipolaris sorokiniana*) of wheat [87], kernel rot (*Aspergillus flavus*) of pre-harvest maize [101], disease of pear caused by *Physalospora piricola* and *Alternaria kikuchiana* [102], brown rot (*Monilinia fruticola*) of peach fruit [103], post-harvest pathogenic fungi (*Rhizopus stolonifer*, *Aspergillus niger*) of grapes [93], anthracnose (*Plasmopara viticola*) and downy mildew (*Elsinoe ampelina*) of grapevines [104], and downy mildew (*Sclerospora graminicola*) of pearl millet [105].

Furthermore, the antifungal activity of CHT was also documented in vivo in several plant–pathogen systems like in rice against *Rhizoctonia solani* [106], in potato against *Phytophthora infestans* [107], in tomato against *Fusarium oxysporum* [108], in pepper against *Phytophthora capsici* [89], in tobacco against *Phytophthora parasitica* [96], in strawberry and in grapevine against *Botrytis cinerea* [109,110], and in dragon fruit against *Colletotrichum gloeosporoides* [111]. It also controls *Penicillium* spp. [95], *Puccinia* spp. [112], and *Colletotrichum* spp. [113], which causes diseases in a wide variety of crops. These studies have demonstrated that CHT is fungistatic against necrotrophic and biotrophic pathogens. Therefore, we enlisted the antifungal efficacy of CHT obtained from numerous studies on different crops, fruits and vegetables in Table 2.

**Table 2.** Effects of CHT on fungal plant pathogens.

Plant Species	Fungi	Mode of Application	References
Rice ( <i>Oryza sativa</i> )	<i>Magnaporthe oryzae</i>	In vitro	[114]
	<i>M. oryzae</i>	In vivo	[115]
	<i>Rhizoctonia solani</i>	In vitro and In vivo	[106]
Jute ( <i>Corchorus olitorius</i> )	<i>Macrophomina phaseolina</i>	In vivo	[116]
Maize ( <i>Zea mays</i> )	<i>Aspergillus flavus</i>	Pre-harvest treatment	[101]
	<i>Bipolaris sorokiniana</i>	In vivo	[87]
	<i>Fusarium graminearum</i>	In vivo	[117]
Wheat ( <i>Triticum aestivum</i> )	<i>F. graminearum</i>	In vivo	[118]
	<i>Sclerospora graminicola</i>	Post-harvest treatment	[105]
Pearl millet ( <i>Pennisetum glaucum</i> )	<i>Sclerospora graminicola</i>	Post-harvest treatment	[105]
Soybean ( <i>Glycine max</i> L.)	<i>Heterodera glycines</i>	In vivo	[119]
Cowpea ( <i>Vigna unguiculata</i> )	<i>F. oxysporum</i> f. sp. <i>tracheiphilum</i>	In vivo	[91]
Tobacco ( <i>Nicotiana tabacum</i> )	<i>Phytophthora parasitica</i>	In vitro and In vivo	[96]
Cherry tomato ( <i>Solanum lycopersicum</i> var. <i>cerasiforme</i> )	<i>Botrytis cinerea</i>	Post-harvest treatment	[98]



Table 2. Cont.

Plant Species	Fungi	Mode of Application	References
Tomato ( <i>S. lycopersicum</i> )	<i>F. oxysporum</i>	In vivo	[108]
	<i>F. oxysporum f. sp. radialis-lycopersici</i>	In vivo	[120]
	<i>F. oxysporum f. sp. radialislycopersici</i>	In vivo	[121]
	<i>F. oxysporum f. sp. lycopersici</i>	In vivo	[9]
	<i>Alternaria solani</i>	In vivo	[10]
	<i>P. infestans</i>	In vivo	[97]
	<i>F. ox f. sp. lycopersici</i> <i>F. solani</i> <i>F. oxf. radialis. lycopersici</i> <i>A. solani</i> <i>P. infestans</i> <i>R. solani</i> <i>Sclerotium rolfsii</i>	In vitro	[90]
Potato ( <i>S. tuberosum</i> )	<i>F. semibaticum</i> <i>P. infestans</i> <i>A. solani,</i> <i>R. solani</i>	In vitro	[90]
	<i>P. infestans</i>	In vivo	[122]
	<i>P. infestans</i>	In vivo	[107]
Green bean ( <i>Phaseolus vulgaris</i> L.)	<i>F. oxysporium</i> <i>F. solani</i> <i>R. solani</i> <i>S. rolfsii</i> <i>Sclerotina sclerotiorum</i> <i>B. cinerea</i> <i>Macrophomina phaseolina</i>	In vitro	[90]
	<i>F. solani</i> <i>R. solani</i>	In vivo	[123]
Cucumber ( <i>Cucumis sativus</i> L.)	<i>Colletotrichum spp.</i>	Foliar spraying	[113]
	<i>B. cinerea</i>	Foliar spray	[124]
	<i>Sphaerotheca fuliginea</i>	In vitro	[125]
	<i>Phytophthora capsici</i>	In vivo	[126]
Pepper ( <i>Piper nigrum</i> )	<i>P. capsici</i>	In vivo	[89]
Chili pepper ( <i>Capsicum annuum</i> )	<i>Colletotrichum capsici</i>	In vivo	[127]
	<i>P. capsici</i>	In vivo	[128]
Chilli ( <i>Capsicum frutescence</i> L.)	<i>C. capsici</i>	In vivo	[66]
Eggplant ( <i>Solanum melongena</i> )	<i>Ralstonia solanacearum</i>	In vitro	[129]
Papaya ( <i>Carica papaya</i> )	<i>C. gloeosporioides</i>	In situ	[130]
	<i>C. gloeosporioides</i>	Post-harvest treatment	[131]
Carrot ( <i>Daucus carota</i> )	<i>S. sclerotiorum</i>	In vitro	[132]

Table 2. Cont.

Plant Species	Fungi	Mode of Application	References
Grapevine ( <i>Vitis vinifera</i> )	<i>Elsinoe ampelina</i> <i>Plasmopara viticola</i>	Post-harvest treatment	[104]
	<i>B. cinerea</i>	In vitro and In vivo	[109]
	<i>B. cinerea</i> <i>P. viticola</i>	In vivo	[133]
Celery ( <i>Apium graveolens</i> )	<i>Fusarium oxysporum</i> f. sp. <i>apii</i>	In vivo	[134]
Strawberry ( <i>Fragaria</i> × <i>ananassa</i> )	<i>Rhizopus stolonifer</i> <i>B. cinerea</i>	Post-harvest treatment	[135]
	<i>B. cinerea</i>	Pre-harvest treatment	[110]
	<i>Sphaerotheca macularis</i>	In vivo	[136]
Dragon fruit ( <i>Hylocereus undatus</i> )	<i>C. gleosporioides</i>	In vivo	[111]
Kiwifruit ( <i>Actinidia deliciosa</i> )	<i>B. cinerea</i>	Post-harvest treatment	[137]
Pear ( <i>Pyrus communis</i> )	<i>A. kikuchiana</i> <i>P. piricola</i>	In vitro and Post-harvest treatment	[102]
	<i>B. cinerea</i>	Post-harvest treatment	[137]
Peach ( <i>Prunus persica</i> )	<i>Monilinia fructicola</i>	Post-harvest treatment	[103]
	<i>B. cinerea</i>	Post-harvest treatment	[137]
Banana ( <i>Musa acuminata</i> )	<i>P. viticola</i>	In vivo	[138]
	<i>Colletotrichum</i> sp. <i>Fusarium</i> sp.	Post-harvest treatment	[139]
	<i>C. gloeosporioides</i>	Post-harvest coating	[140]
Mango ( <i>Mangifera indica</i> )	<i>C. gloeosporioides</i>	Post-harvest coating	[141]
	<i>C. asianum</i> <i>C. dianesei</i> <i>C. fructicola</i> <i>C. tropicale</i> <i>C. karstii</i>	Post-harvest coating	[142]
	<i>Penicillium italicum</i> <i>P. digitatum</i>	Post-harvest coating	[143]
	<i>Rhizopus stolonifer</i> <i>Aspergillus niger</i>	Post-harvest treatment	[93]
	<i>B. cinerea</i>	Pre-harvest treatment	[144]
Pomegranate ( <i>Punica granatum</i> L.)	<i>Botrytis</i> spp. <i>Penicillium</i> spp. <i>Pilidiella granati</i>	Post-harvest coating	[95]
Avocado ( <i>Persea americana</i> mill.)	<i>C. gloeosporioides</i>	Post-harvest treatment	[16]
Soursop ( <i>Annona muricata</i> L.)	<i>C. gloeosporioides</i>	Post-harvest treatment	[139]



Table 2. Cont.

Plant Species	Fungi	Mode of Application	References
Jackfruit ( <i>Artocarpus heterophyllus</i> L.)	<i>Rhizopus</i> sp.	Post-harvest treatment	[139]
Sweet cherry ( <i>Prunus avium</i> )	<i>Monilinia fructicola</i> <i>B. cinerea</i>	Post-harvest treatment	[145]
Tea ( <i>Camellia sinensis</i> L.)	<i>Exobasidium vexans</i>	Foliar spraying	[146]
Scots Pine ( <i>Pinus sylvestris</i> L.)	<i>Fusarium</i> spp.	In vivo	[81]
Date palm ( <i>Phoenix dactylifera</i> )	<i>F. oxysporum</i>	In vivo	[92]
Oil palm ( <i>Elaeis guineensis</i> )	<i>Ganoderma boninense</i>	In vivo	[147]
Peanut ( <i>Arachis hypogaea</i> )	<i>Puccinia arachidis</i>	In vivo	[112]
Groundnut ( <i>Arachis hypogaea</i> )	<i>Phaeoisariopsis personata</i>	Foliar spraying	[148]

#### 4.2. Antibacterial Activity of CHT

Like fungi, bacteria are also extremely sensitive to CHT and its derivatives. Most antibacterial CHT reports relate to human bacterial diseases caused by *Staphylococcus aureus*, *Escherichia coli* and other *Bacillus* species. While CHT shows bactericidal activities toward a range of human diseases caused by bacteria, it might be anticipated that CHT can protect plants from bacterial infections. Some researchers recently found that CHT has strong in vitro and in vivo antibacterial activities towards different plant pathogenic bacteria, like *Xanthomonas* spp. [149,150], *Pseudomonas* spp. [151,152], *Streptomyces scabies* [153], *Burkholderia seminalis* [154], *Acidovorax* spp. [155,156]; *Ralstonia solanacearum* [157], and *Staphylococcus aureus* [152].

Foliar application of a commercial CHT formulation (Armour-Zen®) was capable of reducing the occurrence of *Xanthomonas vesicatoria* in vitro and in vivo growing tomato plants [150]. Li et al. [149] also reported significant antibacterial activity towards leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) and leaf blight (*Xanthomonas oryzae* pv. *oryzae*) of rice. CHT solutions have considerably decreased disease incidence and lesion length of broccoli that were inoculated with *Pseudomonas fluorescens* [151]. A commercial formulation of CHT known as Elexa strongly protected cucumber from bacterial angular leaf spot damage caused by *Pseudomonas lachrymans* [158]. The inhibitory activity of CHT against bacteria varied with concentration of CHT used [155], molecular weight [159], bacterial type (Gram-positive and Gram-negative) [160], bacterial surface and cell wall composition structure [161], solvent type [162]; period of incubation and abiotic factors [163]. From these findings, it is apparent that CHT can be utilized as a potential control agent for plant diseases caused by bacteria. Bacterial plant pathogens that are inhibited by CHT are enlisted in Table 3.

**Table 3.** Antibacterial effects of CHT on bacterial plant pathogens.

Plant Species	Bacteria	Mode of Application	References
Rice ( <i>Oryza sativa</i> )	<i>Acidovorax avenae</i> subsp. <i>avenae</i>	In vitro	[155]
	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> <i>X. oryzae</i> pv. <i>oryzicola</i>	In vitro and In vivo	[149]
Tomato ( <i>Solanum lycopersicum</i> )	<i>Ralstoniasolanacearum</i>	In vivo	[164]
	<i>X. vesicatoria</i>	In vivo	[165]
	<i>X. vesicatoria</i>	In vitro and in vivo	[150]
Potato ( <i>S. tuberosum</i> )	<i>Streptomyces scabies</i>	In vivo	[153]
	<i>Ralstonia solanacearum</i>	In vitro and in vivo	[157]
Broccoli ( <i>Brassica oleracea</i> var. <i>italica</i> )	<i>Pseudomonas fluorescens</i>	In vitro and In vivo	[151]
Cucumber ( <i>Cucumis sativus</i> )	<i>P. syringae</i> pv. <i>lachrymans</i>	In vivo	[158]
Kiwifruit ( <i>Actinidia chinensis</i> )	<i>P. syringae</i> pv. <i>actinidiae</i>	In vitro	[166]
Apricot ( <i>Prunus armeniaca</i> )	<i>Burkholderia seminalis</i>	In vitro	[154]
Watermelon ( <i>Citrullus lanatus</i> )	<i>A. citrulli</i>	In vitro	[156]
Banana ( <i>Musa acuminata</i> )	<i>P. aeruginosa</i> <i>Staphylococcus aureus</i>	In vitro	[152]
Apple ( <i>Malus domestica</i> )	<i>P. aeruginosa</i> <i>S. aureus</i>		
Poinsettia ( <i>Euphorbia pulcherrima</i> )	<i>Xanthomonas</i> spp.	In vitro	[167]

#### 4.3. Antiviral Activity of CHT

Chitosan has been demonstrated to suppress a few plant viral diseases. Nevertheless, it has yet to be proven that CHT inactivates viruses directly, which in itself would seem impossible, as viruses do not have chitin or associated polysaccharides. It has been proved that CHT inhibits the systemic proliferation of viroids and viruses across the plant and enhances the host's hypersensitive reactions to infection [87]. CHT's ability to inhibit viral plant infections is not dependent on type of virus, as CHT impacts the plant itself by triggering resistance to viral diseases [168]. Chirkov et al. [169] observed that CHT application on potato plants inoculated with potato virus X (PVX) displayed resistance to PVX virus. In addition, CHT-treated tomato plants displayed resistance to tomato mosaic virus, and also improved their vegetative growth [170]. Moreover, the formulation of CHT with plant growth-promoting rhizobacteria exerted leaf curl virus resistance in tomato plants [171]. CHT has also been found to be efficient towards the suppression of squash mosaic virus (SMV) [172]. CHT protected different plant species from systemic and local infection [169] when virus particles were applied on plant leaves either by inoculating or spraying. The effects of CHT on various phyto-pathogenic viruses are listed in Table 4.

**Table 4.** In vivo antiviral activities of CHT.

Plant Species	Viruses	References
Tomato ( <i>Lycopersicon esculentum</i> )	PVX, TYLCV	[173]
	ToLCV	[171]
	PSTV, TMV	[168]
Potato ( <i>Solanum tuberosum</i> )	PVX	[169]
	PVY	[168]
Bean ( <i>Phuseolus vulgaris</i> )	AMV	[173]
	AMV, BGMV, PSV, TNV, BYMV, TMV, BCMV	[168]
	BCMV	[11]
Cucumber ( <i>Cucumis sativus</i> )	SMV	[172]
Globe amaranth ( <i>Gomphrena globosa</i> L.)	PVX	[168]
Pea ( <i>Pisum sativum</i> )	AMV, PSV	[173]
Quinoa ( <i>Chenopodium quinoa</i> )	TNV	[173]
	CMV, TNV, AMV	[168]
Tobacco ( <i>Nicotiana tabacum</i> )	TMV	[174]
	TNV	[175]
	TMV	[170]
	PSV	[173]
Stramony ( <i>Datura stramonium</i> L.)	FMV, TMV	[168]
Plum ( <i>Prunus domestica</i> )	PPV	[176]

Viruses: PVX, potato virus X; TYLCV, tomato yellow leaf curl virus; ToLCV, tomato leaf curl virus; PSTV, potato spindle tuber viroid; TMV, tobacco mosaic virus; PVY, potato virus Y; AMV, alfalfa mosaic virus; BGMV, bean goldish mosaic virus; PSV, peanut stunt virus; TNV, tobacco necrosis virus; BYMV, bean yellow mosaic virus; MV, squash mosaic virus; BCMV, bean common mosaic virus; CMV, cucumber mosaic virus; FMV, figwort mosaic virus; PPV, plum pox virus.

#### 4.4. Nematicidal Activity of CHT

A range of studies has suggested, from the 1980s onward, that CHT is useful in controlling the population of plant pathogenic nematode [177]. Application of CHT in soil promotes the multiplication of chitinolytic microorganisms that degrade chitin containing the organ of plant parasitic nematodes, and reduces egg hatching and the viability of larvae and adults belonging to *Meloidogyne javanica* [178], *Meloidogyne arenaria* [177], and *Heterodera schachtii* [179]. Due to the high nitrogen content in CHT, higher emissions of ammonia can also create toxicity to nematodes [180]. CHT showed elicitor activity by activating mechanisms of systemic and local resistance of tomato plants toward the root-knot nematode *Meloidogyne incognita*. CHT with low molecular weight controls *M. Incognita* in a better way [180]. Pinewood nematode (*Bursaphelen chusxylophilus*), a stem nematode, is managed by CHT-based nanoparticles of avermectin [181]. CHT improves the parasitism of *Meloidogyne javanica* eggs by *Pochonia chlamydosporia*, and also increases the differentiation of appressorium in *Pochonia chlamydosporia* [178]. In addition, Westerdahl et al. [179] reported that chitosan's control level of nematodes on walnuts and potatoes was higher compared to synthetic nematicide 1,3-dichloropropene. The effects of CHT on various plant pathogenic nematodes are summarized in Table 5.

**Table 5.** Nematicidal effect of CHT on nematode plant pathogens.

Plant Species	Nematodes	Mode of Application	References
Rice ( <i>Oryza sativa</i> )	<i>Aphelenchoides besseyi</i>	In vivo	[182]
	<i>Meloidogyne incognita</i>	In vivo	[180]
Tomato ( <i>Solanum lycopersicum</i> )	<i>M. incognita</i>	In vitro and in vivo	[178]
	<i>M. incognita</i>	In vitro and Fertigation	[183]
	<i>M. incognita</i>	Fertigation	[179]
	<i>Meloidogyne</i> spp.	In vivo	[184]
	<i>M. javanica</i>	Fertigation	[178]
	<i>M. hapla</i>	Fertigation	[185]
Potato ( <i>S. tuberosum</i> )	<i>M. chitwood</i>	Fertigation	[179]
Eggplant ( <i>S. melongena</i> )	<i>M. incognita</i>	Fertigation	[186]
Brussels sprouts ( <i>Brassica oleracea</i> )	<i>Heterodera schachtii</i>	Fertigation	[179]
Valencia orange ( <i>Citrus sinensis</i> Valencia)	<i>Tylenchulus semipenetrans</i>	Fertigation	[187]
Walnut ( <i>Juglans regia</i> )	<i>Pratylenchus vulnus</i>	Fertigation	[179]
Groundnut ( <i>Arachis hypogaea</i> L.)	<i>M. arenaria</i>	Fertigation	[177]
Pinewood ( <i>Pinus</i> sp.)	<i>Bursaphelen chusxylophilus</i>	Fertigation	[12]
	<i>B. chusxylophilus</i>	Fertigation	[181]

## 5. Mechanism of Actions of Chitosan Biopolymer

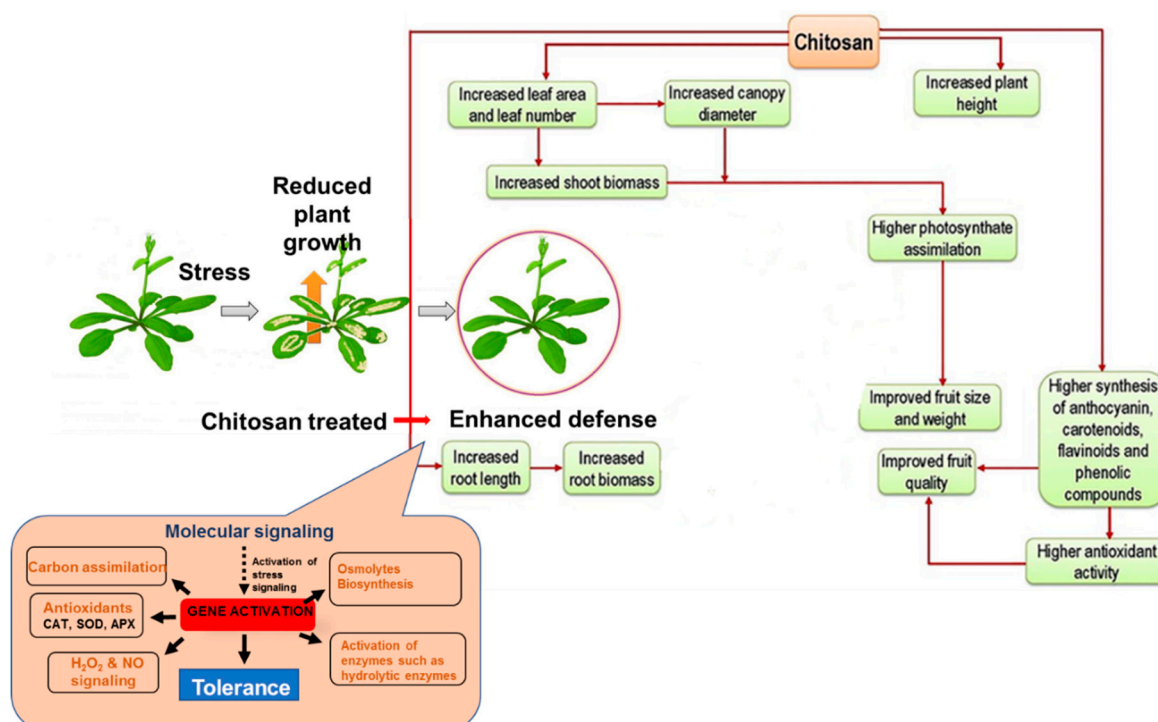
### 5.1. CHT as a Plant Growth Promoter

Numerous findings have been documented on various crops regarding the application of CHT as in vitro, in vivo, soil application, pot application and biofertilization to promote plant growth (Figure 1). CHT facilitates plant growth by increasing the uptake and availability of water and important nutrients by adjusting osmotic pressure in the cells [17]. For the past decade, signaling mechanisms of CHT and its derivatives were studied to control plant growth and development processes. Initial findings revealed that CHT helped activate the hydrolytic enzymes needed to degrade and mobilize reserve food materials including starch and protein [188]. CHT can promote the division of root cells by activating plant hormones including auxin and cytokinin that further lead to increased nutrient intake [189,190]. Other potential contributions are higher seed germination, enhanced seedling growth and development, and activation of antioxidant enzymes to prevent the potential damage by the reactive oxygen species (ROS) at the time of seed germination [62,74,188].

Amin et al. [191] reported that plant-growth-enhancing activities of CHT can be directly linked to impacts on plant physiological mechanisms, including nutrient absorption, cell division, cell elongation, enzymatic activation and synthesis of protein. According to Batool and Asghar [70], *Carum copticum* seeds primed with varied concentrations of CHT led to an increase in percentage of germination, rate of germination, vigor index of seedling, hypocotyl, and dry weight and radical length compared to control. Shao et al. [58] observed that maize seeds soaked with CHT significantly enhanced the percentage of germination. Seed priming with CHT Nanoparticles (NPs) stimulated seed germination percentage and the vigor index of maize, tomato, and chickpea, leading to the early establishment of healthy seedlings [59,62,74].

Zeng and Luo [192] stated that CHT has an excellent property of forming a semi-permeable film on the seed surface that can retain the moisture of the seed and absorb additional moisture from the

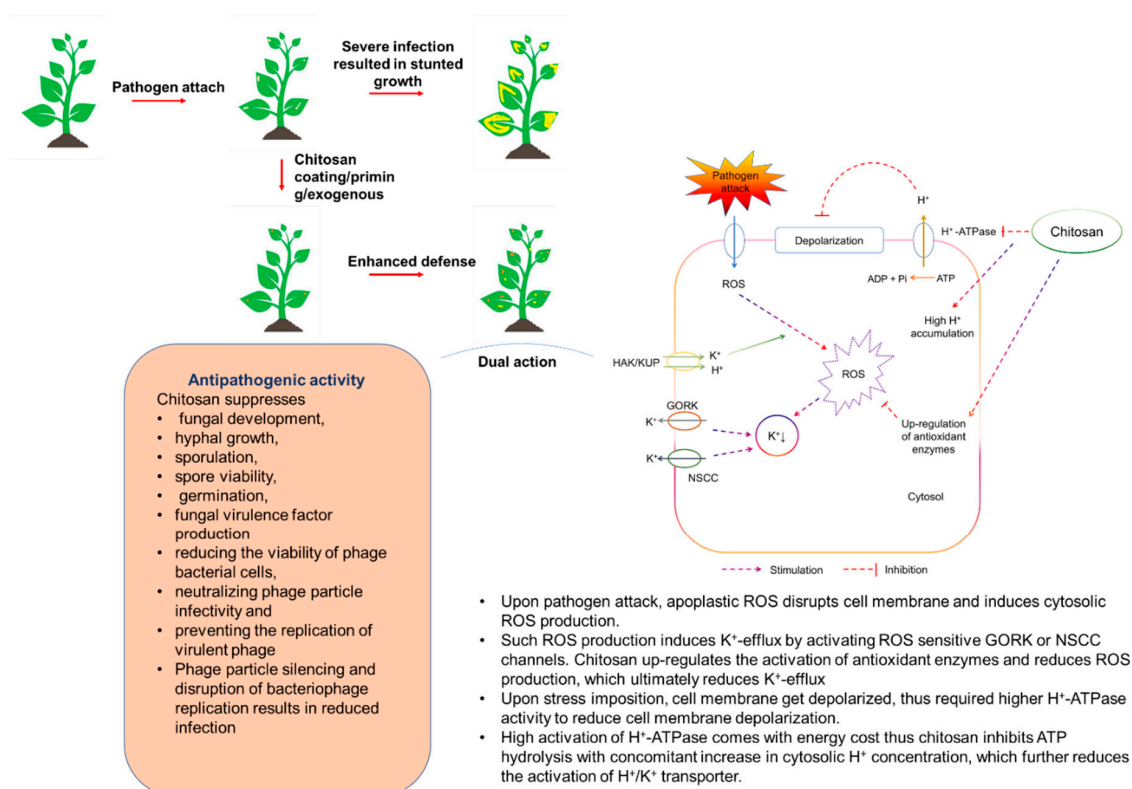
soil, thereby promoting seed germination. Treatment of maize seed with Cu-chitosan NPs controlled the synthesis of hydrolytic enzymes like protease and  $\alpha$ -amylase, and strengthened their activities. Increased activity of protease and  $\alpha$ -amylase led to the rapid mobilization and degradation of preserved food, which resulted in increased germination and SVI of maize [59]. CHT NPs also significantly enhanced the biophysical properties, such as the nutrient intake and net rate of photosynthesis, which contributed to coffee seedling growth promotion. Positively charged nano-sized CHT can easily penetrate into plant cells or adhere to plant surfaces and enhance seed germination and biophysical properties. CHT also increased crop yield substantially by improving the index of photosynthesis by enhancing stomatal function and chlorophyll content. The polycationic CHT raises stomatal cells' osmotic pressure, resulting in increased stomatal opening and  $\text{CO}_2$  integration. In addition, CHT also enhances the biomass content of the leaf area and nitrogen fixation nodules of soybean [193].



**Figure 1.** An overview of chitosan-mediated plant growth regulation under stress conditions.

## 5.2. CHT as Plant Disease Suppressor

From the discovery of chitosan's broad-spectrum antimicrobial properties, considerable interest in this polymer and its derivatives has arisen in recent years. Several research findings have undoubtedly proven their potential application in controlling plant diseases in agriculture (Figure 2). Research related to unraveling the mechanism involved with the antimicrobial activity of microbiocides is an essential step in the developmental process. Nonetheless, the actual mechanisms of the antimicrobial activities of CHT and similar natural products are still unclear, which somehow restricts their use. Over the past decades, multiple modes of action have been suggested to explain CHT's antimicrobial activity. Based on the findings from current studies, some concrete evidence regarding CHT's antimicrobial mechanism and its potential to induce plant defense responses is now available. These growing proofs confirm that the CHT and CHT-derived products have dual modes of action, i.e., they suppress pathogen growth and alter the plant defense responses [194–196].



**Figure 2.** An overview of chitosan-mediated disease suppression in plants.

There are numerous reactive amino groups present in the structure of polycationic CHT polymer, which can be protonated, so the polymer can bear a net positive charge. The existence of reactive amino groups and positive charge is hypothesized to be the basis of CHT's direct antimicrobial actions. The electrostatic interaction of negatively charged cell membranes of microbes with positively charged CHT and its derivatives leads to agglutination, destruction, and alteration of the cell membrane's intracellular ultrastructure, which induces death of the organism [197,198]. The Gram-positive bacterial cell wall comprises a thick teichoic acid-rich peptidoglycan layer that is negatively charged due to the existence of phosphate groups within the structure, as lipopolysaccharides convey a highly negative charge to the bacterial surface in Gram-negative bacteria. In addition, the fungal cell membrane and viral envelope contain similar negatively charged substances (proteins, and glycoproteins). Recently, Lopez-Moya et al. [114] reported that CHT induces plasma membrane permeabilization of *Magnaporthe oryzae* fungus of rice, and influences NADPH oxidase-dependent synthesis of ROS, important for fungal pathogenicity.

CHT can also affect the cell membrane structure by interfering with proteins in bacterial cell membrane (Figure 2) [199]. Therefore, it is assumed that membrane proteins may be the target molecules for the action of CHT on cell surfaces. The potassium ion efflux has been reported as an early cell response to the presence of certain cationic molecules. A rapid potassium efflux was reported, which was dependent on the concentration of CHT. Moreover, CHT has a strong inhibitory effect on  $H^+$ -ATPase activity in the *Rhizopus stolonifer* plasma membrane. Decreased activity of  $H^+$ -ATPase may result in proton accumulation within the cell, leading to the inhibition of chemical transport driven for the exchange of  $H^+/K^+$  [200]. The positively charged CHT molecules thus associate with negatively charged pathogenic surfaces, which damage the structure of the cell. This damage causes significant modifications to the surface of the cell and improves the membrane permeability, resulting in intracellular molecules' leakage and eventually impairing vital activities of the pathogen [201–203].

An essential role of the cell wall and cell membrane is to protect the internal substances so that they do not spill outside of the cell [204]. Chung and Chen [205] stated that CHT responded to both



the cell wall and cell membrane, and inhibited the bacterial growth through a two-step synchronous mechanism: the initial cell wall separation from its cell membrane, accompanied by cell membrane destruction. The function of the pathogen genetic material may be interfered with by CHT. The nucleic acid (DNA or RNA) contains negatively charged phosphate groups in the primary chain. The CHT can penetrate the cell wall and attach to the negatively charged DNA, thus blocking the mRNA and the essential pathogenic proteins' synthesis [198,206].

It is believed that CHT with lower molecular weight can move through the cell wall of bacteria [207,208], degrade intracellular materials from the colloidal phase to degeneration and flocculation, interfere with normal physiological and metabolic activities of bacteria or interact with genetic materials directly [209,210], and then suppress the bacterial reproduction, which leads to micro-organisms' death. It is assumed that CHT may bind with DNA and inhibit messenger RNA (mRNA) synthesis via penetration to the microorganism nuclei and interfere with mRNA and protein synthesis [201,207].

The mechanism of action of CHT could also be linked to its capacity to chelate certain necessary nutrients, trace elements, and metal ions required for bacterial and fungal growth [201,211]. Another potential mode of CHT's antibacterial effect is depositing on pathogen surface and forming a thick layer of polymer. This thick polymer film inhibits the process of nutrient flow and microbial metabolism that are important to their survival [194]. Plants also defend themselves against pathogens by creating a remarkable array of structural, chemical and protein-based safeguards designed to identify and arrest invasive pathogens before they can cause significant damage [212].

However, CHT only displays its antibacterial effect in an acidic medium, due to its low solubility beyond pH 6.5. CHT's water-soluble derivatives can also be strong candidates, like polycationic biocide, which are soluble in both basic and acidic physiological conditions [213].

The impacts of CHT on the plant–fungal interaction of *Botrytis cinerea* in tomatoes and strawberries have been linked with aflatoxin reduction, phenolic, and phytoalexin precursors elicitation, increased production of chitinases and other plant defense factors [195,214]. In addition to producing phytoalexin, CHT also blocks the production of toxin by *Alternaria alternata* and macerating enzymes by *Erwinia* [215, 216]. The direct interaction of *Aspergillus flavus* with CHT has been documented as generation of hyphal swelling and weakening [93]. The fungistatic properties of CHT against *Rhizopus stolonifer* were linked to its ability to cause morphological modifications in the cell wall [217].

CHT's antiviral activity was found to rely on average polymerization frequency, degree of N-deacetylation, quality of positive charge, and character of the molecule's chemical modifications. CHT is hypothesized to suppress viral infection by triggering a hypersensitive response, reducing the phage bacterial cells' viability, neutralizing phage particle infectivity, and preventing the replication of virulent phage [168]. Phage particle silencing and disruption of bacteriophage replication at the cellular level are the key factors in preventing phage infections by CHT. By its potential to trigger resistance to viral diseases in plants, CHT exhibits an antiviral effect to inhibit the replication of bacteriophages in contaminated microorganism cultures. Imitating the plant's interaction with a phytopathogen, CHT triggers a broad array of protective responses in the plant, which restrict the systemic spread of viroids and viruses throughout the plant, resulting in systemic acquired resistance development [168].

Some studies show that CHT and its derivatives are powerful elicitors and inducers of systemically acquired resistance of plants against a wide array of pathogens. CHT and oligochitosan induce hosts to generate protein, enzymes, and secondary metabolites linked to the protection from pathogens [194, 206,218]. CHT and its derivatives enhance glucanase levels and activity in rice, wheat, tobacco, etc. [219–221]. CHT and its derivatives are also reported to enhance the activity of chitinase, peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase, superoxide dismutase and catalase in wheat, cucumber, tomato, sweet cherries, table grapes, pears, orange, strawberries, *Zanthoxylum bungeanum* and ginseng [194,196,222–225]. The pathogen is inhibited directly or indirectly by all the stated proteins and enzymes. Generally, genes which functionally produce disease resistance are known as pathogenesis-associated genes. Several findings revealed that CHT uses several modes to improve the

function of pathogenesis-associated genes. According to Hoat et al. [226], CHT and chitin significantly triggered the pathogenesis-associated gene expression in oat leaves. It is well known that in the plant defense system, secondary metabolites, including phytoalexins, suberization, lignin and phenolic compounds, perform a major role. The role of CHT in defense-associated secondary metabolites accumulation in plant tissue was reported in the 1980s, since Hadwiger and Beckman [227] noticed that CHT could trigger the accumulation of phytoalexin in soybean pod at a concentration of 0.9 µg mL<sup>-1</sup> in 24 h.

Resistant plants possess the ability to identify plant pathogens quickly to activate the defense mechanism rapidly and fruitfully. Activation of the defensive mechanism is expressed through hypersensitive response (HR) in the infection site and activation of defense in the unaffected part of the plant [228]. The elicitation of HR and systemic acquired resistance (SAR) are regulated by the host and pathogen's genetic background and depends on a complex signal exchange that occurs under the given environmental conditions. Signal transduction mechanisms consist of stimulation of the target receptor on the cell membrane or intracellular site, followed by signal propagation to the target cell by one or more second messengers and the formation of physiological response sequences. Lectin and kinase 1 (CERK1) are two cell membrane receptors that are able to bind CHT and its oligomers [229,230]. CHT also affects the DNA conformation of the plant. Because of the high affinity of DNA backbone to negatively charged phosphates, CHT can modify chromatin by competing with specific nuclear proteins for the DNA binding sites, which in turn block gene transcription [206,226]. It has been reported that ROS, Ca<sup>2+</sup>, nitric oxide (NO), ethylene (ET), jasmonic acid (JA), abscisic acid (ABA) and salicylic acid (SA) all participate in the CHT-mediated signaling pathway [6,194].

From the above-mentioned body of literature, it may be concluded that CHT and its derivatives are capable of directly destroying the pathogen and activating the immune (defense response) system of plants via various signaling processes or through regulatory molecules engaged in signal transduction [231]. The mode of action of CHT in preventing plant infections has not been clearly understood, despite extensive study. Some of the proposed modes of action of CHT against various pathogens are enlisted in Table 6.

**Table 6.** Proposed antimicrobial mechanisms of CHT.

Pathogens	Mechanisms	References
<i>Magnaporthe oryzae</i>	Permeabilized the cell plasma membrane and affected the NADPH oxidase-dependent synthesis of ROS	[114]
<i>Rhizoctonia solani</i>	Disrupted cell plasma membrane, destroyed cell structures and induced defense-associated enzymes activity in plants	[106]
<i>Saccharomyces cerevisiae</i>	Destroyed synthesis of protein, and integrity of cell membrane	[232]
<i>Candida albicans</i>	Disrupted the integrity of cell wall and intra-cellular ultrastructure	[233]
<i>Beauveria bassiana</i> <i>Pochonia chlamydosporia</i> <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i>	Fluidity of the cell membrane determines the vulnerability of fungi to CHT	[234]
<i>F. oxysporum</i> <i>F. solani</i>	Had an attraction for lipids in the plasma membrane	[7]

Table 6. Cont.

Pathogens	Mechanisms	References
<i>Neurospora crassa</i>	Fluidity of the cell membrane determines the vulnerability of fungi to CHT	[234]
	Permeabilized the cell membrane and destroyed cells	[235]
<i>Rhizopus stolonifer</i>	Triggered K <sup>+</sup> efflux and inhibited the activity of H <sup>+</sup> -ATPase	[200]
<i>Aspergillus fumigatus</i> <i>Botrytis cinerea</i> <i>Aspergillus parasiticus</i> <i>Penicillium verrucosum</i> var. <i>verrucosum</i>	Had an attraction for lipids in the plasma membrane	[7]
<i>Alternaria alternata</i>	Chelation of metals	[216]
<i>Bacillus cereus</i>	Blockage of nutrient flow	[236]
<i>Escherichia coli</i>	Disrupted cell membranes and leaked cellular cytoplasm	[237]
	Destroyed plasma membrane structure of cells, and bind to intracellular or extracellular targets	[199]
	Destroyed structure of cells, electrostatic interactions, induced enzyme and nucleotide leakages	[205]
	Disrupted cell membrane structures, and leaked cellular cytoplasm	[238]
	Destroyed cell membrane	[198,203]
	Blockage of nutrient flow	[236]
	Increased cell plasma membrane permeability by CHT-membrane interaction	[239]
<i>Neisseria subflava</i>	Destroyed the integrity of cell wall, and intra-cellular ultrastructure	[233]
<i>Pseudomonas syringae</i>	Electrostatic interactions, disrupted bacterial cell surface and induced morphological alterations	[240]
<i>P. fluorescens</i>	Increased cell plasma membrane permeability by CHT-membrane interaction	[239]
<i>P. aeruginosa</i>	Disrupted outer cell membrane	[198]
<i>Streptococcus sobrinus</i>	Destroyed the integrity of cell wall, and intra-cellular ultrastructure	[233]
<i>Staphylococcus simulans</i>	Electrostatic interactions	[241]
<i>S. aureus</i>	Electrostatic interactions	[241]
	Destroyed cell plasma membrane	[203]
	Disrupted cell plasma membranes and leaked cellular cytoplasm	[237]
	Damaged structures of cell membrane, and bind to intracellular or extracellular targets	[199]
<i>Salmonella typhimurium</i>	Destroyed the outer cell membrane	[198]

## 6. Concluding Remarks and Future Perspectives

Chitosan, a chitin derivative, is the second most widely distributed abundant natural polymer. Over the last decade, the number of uses of CHT and its derivatives has significantly increased. The availability of information on biocompatible and biological characteristics of CHT makes it a potential bioactive substance for agriculture. CHT is a versatile nontoxic compound with multiple modes of action to positively impact plant health. Its application can mitigate the broad use of chemical pesticides, at least in part. To date, there is ample evidence to suggest that plants may achieve improved tolerance to a broad range of pathogenic micro-organisms, and promote growth and development after the application of CHT, suggesting that the utilization of natural elicitors like CHT may be an essential component of sustainable agriculture.

While a lot of work has been done, several issues still remain unclear pertaining to the mechanisms of pathogens' growth inhibition by CHT, inducing plant immunity, accelerating plant growth and development. In that regard, research and development should pay attention to discovering new derivatives of CHT, as their effective chemical alteration might significantly boost its antimicrobial efficacy, improve its chemical and physical characteristics, and enhance its field applicability by ensuring low mammalian toxicity. CHT and its derivatives apparently rely on their molecular weight for the majority of physiological activity and functionality. In addition, further study is needed to confirm whether biopolymers like CHT have the ability to influence physiological processes or metabolism in microbes. Future studies may aim at explaining the real target molecule on the cell membrane, or even other intracellular targets in case of an antimicrobial mechanism of action. Moreover, further investigations are also required for pathogen resistance mechanisms against this polymer.

Therefore, future studies should also concentrate on understanding the details at the molecular levels, which can offer an insight into the unknown biochemical mechanisms of CHT. It may provide significant benefits if gene mutant strains of microbes can be developed to study the antimicrobial mechanisms of CHT. Combined proteome and transcriptome study of known proteins and genes would enhance our knowledge of the complex CHT-mediated signal pathway and allow for improving biotechnological approaches in plant infection control and growth promotion. A better understanding of CHT's mode of action in plants and pathogens would improve the possibility of its effective application. Furthermore, the collaboration and participation of research organizations, government regulatory authorities and industries will be the primary key to the success of CHT use by unraveling its antimicrobial characteristics, innate immunity-induced activities, growth enhancement in plants and biotechnological prospects for sustainable agriculture.

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