Chapter

Chitosan Nanoparticle: Synthesis, Characterization, and Use as Plant Health Materials

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Abstract

Chitosan is a naturally occurring biopolymer having multifaceted applications in agriculture, medicine, food industry, and cosmetics. The association of this natural biopolymer with nanotechnology can produce revolutionary effects in plant protection and agriculture. Nano-chitosan can be fabricated using various methods. However, the green synthesis approach has gained attention in recent years. The green engineered nanoparticles are economical, energetically feasible, and environmentally benign. The biosynthesized nano-chitosan has evolved as a potential plant protection agent. Chitosan nanoparticles possess antifungal, antibacterial, and antiviral properties, and are found to be effective against seed-borne and soil-borne pathogens. Nano-chitosan also behaves as an effector molecule and induces local and systemic defense responses in plants. The mode of action of nano-chitosan involves alterations in membrane permeability, replication, cytoplasmic alterations, induction of defense-related genes, and cell lysis. Furthermore, chitosan nanoparticles can be used for soil improvement and can reduce pest and pathogen attacks, thereby promoting the growth of plants. The authors outline the methods of synthesis and characterization of chitosan nanoparticles, their utilization in plant protection and growth promotion, along with the underlying mechanisms.

Keywords: chitosan nanoparticles, biopolymer, green synthesis, characterization, plant protection

1. Introduction

Agriculture is a primary activity upon which the economic status of a country relies. The produce obtained from agricultural activity serves the purpose of mitigating domestic hunger as well as earning foreign currency. This demands the high production and productivity of crops. However, crop health, quality of production, and productivity are attributed to different biotic and abiotic factors associated with it. It has been already said that 20–40% of crop loss occurs around the globe due to attack of pest and diseases [1] that limits the yield of crops. The detrimental effect of chemical pesticides on Earth has resulted in the development of green strategies, such as sustainable farming, with the use of resistant varieties, biopesticides, nano bioformulations, integrated disease, and pest management.

As the interest of the scientific community shifted from chemocentric to sustainable agriculture, it opened up a vast possibility of exploiting nature-based biodegradable materials with potential biocontrol efficacy for plant disease management. With more intense research in this field led the scientists to speculate that the nano-sized materials may perform excellent activity as compared to the base source used for their synthesis. Therefore, the development of nano bioformulations and their use is encouraged to achieve the goal of sustainable farming. Different biopolymers viz., cellulose, starch, alginate, chitin, and chitosan, are used for the development of new materials with noble functionality and environmental sustainability. Among these, chitosan is the second most abundant biopolymer found in nature which is used widely due to its unique characteristics, such as abundance, large surface-to-volume ratio, biodegradability, biocompatibility, pH sensitivity, non-toxicity, and a safe alternative [2, 3]. Apart from that, nano-chitosan possesses antifungal and antibacterial activity along with other plant growth-promoting traits [4, 5] which makes it promising in several aspects of plant growth and development.

This chapter aims to briefly review the importance, green synthesis, characterization, mode of action, and successful use of chitosan nanoparticles (ChNPs) for the effective management of plant diseases.

2. Importance of chitosan nanoparticles

Chitin and chitosan are the primary components of crustacean shells, such as shrimp, squid, crab, and lobster, the exoskeleton of terrestrial insects viz., honeybees and silkworm, and cell walls of fungi like molds, yeast, and ray fungi, such as *Streptomyces* [6, 7]. Chitosan is a cationic biopolymer obtained by the whole or partial deacetylation of chitin. It is a linear polysaccharide consisting of (1–4)-linked 2-amino-2-deoxy-β-D-glucopyranose obtained after deacetylation of N-acetyl-D-glucosamine [8, 9]. The term chitosan does not specifically indicate a unique compound but a group of co-polymers owing to their degree of deacetylation, polymerization, molecular mass, viscosity, and acid dissociation constant, that is, pka [10]. The chitosan derived from microbial sources is considered as promising as the process underlying can be manipulated to prepare a pure and uniform product with desired specific characteristics [11]. It is a very versatile biopolymer having multifaceted activity in the field of medicine, agriculture, food industry, cosmetics, and sewage treatment (**Figure 1**) [12]. ChNPs are widely used due to their unique polymeric cationic character, absorption enhancing effects, mucoadhesive nature, biocompatibility, and biodegradability. Being a modified linear polysaccharide with varying numbers of free amino groups in their polymeric chain with cationic property, chitosan offers ionic cross-linking of multivalent anions, which is making it a significant biopolymer for the synthesis of nanoparticles [6, 13]. The positively charged ChNPs have more affinity toward the negatively charged biological membrane and site-specific targeting *in vivo* [14]. Chitosan when applied to foliage or soil, can elicit innate defense response within the plant to resist insect and pathogen attack [5] by the production of antifungal hydrolases, phytoalexins, or by inducing structural barriers via the synthesis of lignin-like material [15, 16]. Further, ChNPs significantly have a positive impact on the biophysical properties of the plant. The application of nano-chitosan increases the rate of photosynthesis, induces root nodulation, upregulates nutrient uptake, enhances the rate of germination of seed, and boosts plant vigor [5, 17]. It is widely used in the delivery of fertilizers, micro-nutrients, and pesticides as it ensures

Figure 1.
Applications of nano-chitosan.

slow release of the drugs with enhanced solubility [10]. Moreover, chitosan-mediated genetic transformation is also a successful one as it forms a complex via electrostatic interaction and protects the nucleic acid from nuclease degradation. It gives rise to stably transformed plants as compared to those developed via traditional methods of gene delivery [18, 19].

3. Green synthesis of chitosan nanoparticles

Green synthesis is a novel method of synthesis of NPs using microorganisms, such as bacteria, fungi, actinomycetes, and botanicals [20–25]. It is a bottom-up approach to nanoparticle synthesis which interconnects two disciplines of science, that is, nanotechnology and biotechnology. Biogenic synthesis of nanoparticles is preferred over chemical or physical synthesis methods as it is a safe, environmentally benign, economically and energetically feasible, less time-consuming process, and it makes optimum use of the redox potential of metabolites produced by biological entities to convert the macromolecules to nano form [9, 21, 26–30]. The main principle of the biogenic synthesis of the nanoparticle is based on the redox reactions that occur when the microorganisms/biological entity grabs the metal ion and detoxifies it to element metal through the enzymatic activity of the cell. It can be categorized into intracellular and extracellular synthesis. In intracellular synthesis, the metal ions are transported into the cell and the reduction reaction occurs within the cell cytoplasm, cell wall, and/or periplasmatic space, therefore, the resultant nanoparticles form inside the cell. However, the latter involves the synthesis of nanoparticles on the cell surface via the catalytic activity of reductase enzyme upon the trapped foreign entity [31]. Harvesting of nanoparticles from the cell matrix is a tedious process in intracellular synthesis which is why extracellular synthesis of nanoparticles is preferred mostly. The microbes are often regarded as eco-friendly green nano-factories due to their large-scale production ability of nanoparticles with relative control over their size and shape (regulated by surrounding environmental conditions) with a simpler process of production [26].

In phytofabrication of nanoparticles, generally, the metal ions are added to the plant part extract and then continuously stirred in a magnetic stirrer. The formation of nanoparticles can be confirmed visually when the resultant solution changes its color [30, 32]. It is due to the activity of the antioxidant, such as polyphenol, flavonoids, and phytoalexins, that act upon the metal ion and convert it to nontoxic element metal in nanoform. Therefore, a plant with a higher percentage of natural reducing constituents is desirable for phytofabrication of nanoparticles. *Pelargonium graveolens* L'Her commonly known as the rose geranium plant is a rich source of natural antioxidants. Its leaves and essential oil have several therapeutic applications in the field of pharmacology [33, 34]. El-Naggar et al. [9] mixed an equal volume of chitosan solution and phytoextract of *P. graveolens* and incubated the mixture at 50°C in a rotary shaker. The resultant turbid solution indicating the ongoing redox reaction is then centrifuged at 10,000 × g for 10 min. It was further washed with an acetic acid solution to remove the unreacted chitosan and the ChNPs present in the solution are extracted by subjecting it to freeze drying. Similarly, Boruah and Dutta [5] biogenically synthesized ChNPs from fungal sources rich in chitosan. Isolation of chitosan was done by treating the fungal biomass with a series of alkali and acid treatments under controlled conditions. Initial treatment of fungal biomass with NaOH yields an alkali-insoluble material (AIM) that was further subjected to an acid treatment to extract the fungal chitosan. It was then converted into nano-chitosan by adding 1% TPP solution in a magnetic stirrer.

Guzman et al. [35] synthesized colloidal AgNps by a combination of ultrasonication and chemical reduction methods using gallic acid and chitosan, respectively, and during characterization, they found that as-synthesized gallic acid-chitosan modified silver nanoparticles (GC-AgNps) were monodispersed, spherical shape with an average size of 26.23 ± 9.92 nm, and stable for four weeks without any noticeable change in size. GC-AgNps were found highly effective against *Escherichia coli* even at 1 μg/mL after 120 min of exposure.

4. Characterization of chitosan nanoparticles

The nanoparticle formation is greatly affected by the processing conditions and time. They are characterized on the basis of their surface plasmon resonance, morphology, particle size distribution, zeta potential, functional group analysis, etc., using UV-vis spectrophotometer, electron microscope, dynamic light scattering, Fourier transform infrared (FTIR) spectroscopy [36]. Moreover, atomic absorption spectroscopy is done to study the release profile of Ch-encapsulated nanoparticles.

4.1 Characterization by UV-vis spectroscopy

UV-vis spectroscopy is the confirmatory analysis that ascertains the formation of nanoparticles by surface plasmon resonance. UV-vis absorption spectroscopy is used to examine the optical properties of ChNPs obtained from the commercial production of chitosan showed an absorption band at 330.25 nm, whereas those obtained from biogenic sources exhibited the absorption band at 310–342 nm [5]. Kain and Kumar [37] reported a band at 200–300 nm for the biogenically synthesized ChNPs from common yarrow. Moreover, AbdElhady [38] studied the UV-vis spectrophotometer reading of ChNPs prepared at two different temperatures, that is, 40°C, 60°C, and 80°C, and found that the absorption band was obtained at 356, 348, and 353 nm, respectively.

4.2 Characterization by dynamic light scattering

The main principle of DLS is based on the Brownian movement of particles/molecules present in the solution that results from their collision with the randomly moving solvent particles. A laser beam is passed through the sample, and the fluctuation in scattered light due to the random motion of particles is detected by the photon detector. DLS is used for the measurement of average particle size, particle size distribution, polydispersity index (PDI), and zeta potential. PDI explains the polydispersity or monodispersity of particles in an aqueous medium. PDI value greater than 0.5 represents polydispersity and less than 0.5 normally shows the monodispersity of particles. Generally, monodisperse ChNPs exhibit the PDI value within the range of 0.2–0.4 [2]. Further, the surface charge of nanoparticles, also known as zeta potential, explains the stability of the nanoparticles, which is measured in the range of ±30 mV. The ChNPs show a positive zeta potential value that may vary from 11.2 ± 1.2 mV to 18.7 ± 0.4 mV [5, 39]. Further, the appropriate particle size determined by DLS showed that the ChNPs are nearly spherical in shape with size ranging from 150 to 350 nm [40]. However, Sivakami et al. [41] prepared ChNPs by cross-linking low molecular weighed chitosan with TPP and found a minimum particle size ˂100 nm. Similarly, Boruah and Dutta [5] reported that the biogenically synthesized ChNPs exhibited their size within the range from 78.36 to 300.1 nm. A mean particle size of 50 nm was obtained by Sahab et al. [42] for the chitosan poly acrylic acid nanoparticles.

4.3 Characterization by electron microscopy

The internal and external morphology of the nanoparticles is studied using a transmission electron microscope and scanning electron microscope. Transmission electron microscopy revealed that the ChNPs are often spherical shaped with an amorphous nature [5, 14, 43]. Similarly, scanning electron microscopy studies conducted by many researchers found that the ChNPs are nearly spherical shaped with the smooth external surface [44, 45]. Parida et al. [46] obtained round ChNPs with a 78 nm diameter. Similarly, Kain and Kumar [37] found that the green synthesized ChNPs of common yarrow (*Achillea millefolium*) are smooth surfaced, spherical with a diameter less than 100 nm with a smallest diameter of 4.15 nm.

4.4 Characterization by Fourier transform infrared spectroscopy

FTIR study is conducted to confirm the synthesis of nanoparticles by determining their functional groups. Sample preparation for FTIR is done by gently triturating it with KBr which is then compressed into disks. The compressed disks are scanned against a blank KBr pellet background at 25°C to obtain the FTIR results. For every spectrum, a 32 scan interferogram was collected at transmittance/absorbance mode in the 4000–400 cm^{-1} region [2]. The functional groups of a chitosan nanoparticle consist of amide ($-NH_2$) and hydroxyl ($-OH$) group, $C-H$, $C-N$, $C-O$, and $P{=}O$ stretching [5]. Generally, the FTIR peak at 3000–3500 cm^{-1} attributed to ($-OH$) and ($-NH_2$) is the confirmatory peak for the formation of ChNPs [46]. Sharma et al. [45] obtained a wider peak of the hydroxyl group (3200–3600), which led them to conclude that hydrogen bonding is enhanced in ChNPs when analyzed by FTIR. Choudhary et al. [2] found a band at 3424 cm^{-1} for the synthesized chitosan nanoparticles that represent the stretching vibration of the combined peaks of the amide

($-NH_2$) and hydroxyl ($-OH$) group. Kain and Kumar [37] reported FTIR peaks at 3317.48, 2139.29, and 1638.46 as a confirmation of the formation of ChNPs. Similarly, Boruah and Dutta [5] obtained a strong and broadband at 3250 cm^{-1} signifying the stretching between hydroxyl and amide groups. They have also found other peaks at 2865 cm^{-1}, 1182 cm^{-1}, 1642 cm^{-1}, and 1182 cm^{-1} is attributed to C–H stretching, asymmetric C–O stretching, stretching between C=O and N–H banding, and P=O.

5. Application of chitosan nanoparticles in plant health management

ChNPs have emerged as a potential antimicrobial agent and found effective against numerous phytopathogens. Nano-chitosan is reported to be effective against seed-borne as well as against soil-borne pathogens. It behaves as an elicitor of plant defense responses, inducing both local and systemic defense responses. Thus, nano-chitosan can be used as a plant health material by protecting the plant from biotic and abiotic stresses and by promoting the growth of the plants.

ChNPs possess a greater affinity toward the membrane of microorganisms and can easily penetrate the pathogen's cell [47]. The smaller size and greater surface area of ChNPs increase the antimicrobial efficiency of chitosan biopolymer. Several studies confirmed ChNPs as an effective plant health management agent due to their dual role as plant protection and plant growth stimulating agents.

ChNPs have been known to possess antifungal properties against numerous phytopathogenic fungi. Nano-chitosan exhibit greater efficacy as compared to its bulk counterpart as NPs can negotiate cell wall and cell membranes more effectively due to their unique physico-chemical properties. It can effectively inhibit the development of phytopathogenic fungi at any stage of their life cycle. Chitosan can completely inhibit spore germination, germ tube elongation, and mycelial growth of fungi [48], and it can penetrate the cell membrane by plasma membrane permeabilization and results in cell lysis [49]. Boruah and Dutta [5] biogenically synthesized chitosan nanoparticles using four different fungal sources *viz.*, *Beauveria bassiana, Fusarium oxysporum, Trichoderma viride,* and *Metarhizium anisopliae. In vitro* assay suggested that synthesized ChNPs in combination with *T. asperellum* was effective in suppressing mycelial growth of soil-borne fungal pathogens *viz.*, *Rhizoctonia solani, Fusarium oxysporum,* and *Sclerotium rolfsii.* Abdel-Rahman et al. [50] studied the efficacy of chitosan (Ch) (2 and 4 g/L) as well as ChNPs (0.2 and 0.4 g/L) against blue rot disease of apples caused by *Penicillium expansum*. They observed that ChNPs performed better than compared to their bulk counterpart for both natural and artificial infections. Also, the fruit quality parameters, such as firmness, titratable acidity, and total soluble solids, were kept intact. The expression of defense-related genes *viz.*, chitinase, β-1,3-glucanase, peroxidase, phenylalanine ammonia lyase-1 (PAL1), xyloglucan endotransglycosylase (XET), and pathogenesis-related protein (PR8), were also upregulated indicating the development of systemic acquired resistance in plants against the pathogen. Saharan et al. [51] synthesized ChNPs using the ionic gelation method and its efficacy was determined against phytopathogenic fungi *viz.*, *Macrophomina phaseolina*, *Alternaria alternata,* and *Rhizoctonia solani*. They observed a decline in the radial growth of the fungi in a dose-dependent manner. Muthukrishnan and Ramalingam [52] synthesized ChNPs biogenically mediating *Penicillium oxalicum*. The nanomaterial was found effective against *Fusarium oxysporum ciceri*, *Pyricularia grisea,* and *Alternaria solani* with the rate of inhibition 87%, 92%, and 72%, respectively. Also, seed treatment with ChNPs exhibited positive morphological effects, such as enhanced germination percentage, seed vigor index, and biomass content in chickpeas. The efficacy of nanomaterials depends on their size, charge, and permeability through biological membranes. Again, the *in vivo* assay conducted under detached leaf condition observed 100% suppression of blast disease symptoms when treated with ChNPs prepared using the ionic gelation method [53]. Kheiri et al. [54] synthesized ChNPs from chitosans of different molecular weight and observed their antifungal activity against *Fusarium graminearum* causing fusarium head blight in wheat. The dynamic light scattering analysis showed a variable size of synthesized nanomaterials (180.9, 339.4, 225.7, and 595.7 nm). The inhibitory effect of these NPs was tested at different concentrations and maximum mycelial growth reduction (77.5%) was observed at 5000 ppm. The results obtained from greenhouse trials indicated a decline in the area under the disease progress curve (AUDPC) in NP-treated plants.

The reports of ChNPs as an antibacterial agent against plant pathogenic bacteria is very scarce and need further thrust in this domain. ChNPs were found effective against *Ralstonia solanacearum* causing bacterial wilt of tomato and potato. *In vitro* experiment indicated an increase in the inhibition zone with increasing concentration of nano-chitosan and found highest at 200 μg/ml concentration. *In vivo* assay revealed foliar application of nano-chitosan led to a decline in the disease incidence and severity in bacterial wilt-infected tomato and potato plants [55]. ChNPs directly interact with the bacterial cell wall and may cause modification in the external shape, loss of flagella, and lysis of the cell. The RAPD-PCR results showed differences in the genotype of treated *Ralstonia solanancearum* as compared to the genotype of untreated isolates [55]. The antibacterial activity of Cu-chitosan nanoparticles against *Pseudomonas syringae* pv. *glycinea* causing bacterial blight of soybean was reported by Choudhary et al. [56]. Concentration of 1000 ppm was found most effective in controlling the bacterial pathogen. An *in vitro* assessment was conducted with ChNPs and chitosan nanocomposites with lime essential oil and thyme critical oil against *Pectobacterium carotovorum.* The results indicated that chitosan nanocomposites with thyme essential oil were effective in producing an inhibition zone [57]. ChNPs were also observed to possess potentially high antibacterial activity against *P. fluorescens* and *Erwinia carotovora* causing bacterial soft rot [58]. Oh et al. [8] reported the antibacterial activity of ChNPs against phytopathogenic bacteria *viz., E. carotovora* subsp. *carotovora* and *X. campestris* pv. *vesicatoria.* Santiago et al. [59] biogenically synthesized Ch-derived NPs containing AgNPs and observed its antibacterial efficacy against *R. solanacearum* causing bacterial wilt of tomato. They found Ag-NP entrapped chitosan as a suitable alternative to chemical bactericides. Cs/TiO_2NPs were found effective against the most dreaded bacterial pathogen of rice *viz*., *X. oryzae* pv. *oryzae* [60].

The chitosan biopolymer was found to inhibit the systemic propagation of viruses and virus-like organisms in infected plants and induce a host hypersensitive response against the viral pathogen [61–63]. The molecular weight of chitosan affects the degree of suppression of viral infections [64]. However, none of the studies has practically proved the ability of the chitosan molecule to absolutely inactivate the virus particles. Most of the studies have reported the inactivation of viral replication that prevents the multiplication and subsequent spread of the virus particles systemically. It may be hypothesized that ChNPs having a smaller size and greater surface area can easily penetrate into host tissues and tightly binds with nucleic acid causing selective inhibition and ramification of virus particles. Lu et al. [65] reported inhibition of TMV in tobacco pants when oligochitosan ($50\ \mu g\ ml^{-1}$) was applied 24 h before inoculation. The epidermal cells of tobacco leave treated with oligochitosan showed

Chitosan nanomaterial	**Application**	**Target pathogen**	**Effect**	**Reference**
Chitosan nanocomposite with *T. asperellum*	Antifungal	*Rhizoctonia solani, Fusarium oxysporum* and *Sclerotium rolfsii*.	Reduction in mycelial growth	[5]
Chitosan NPs	Antifungal	*A. alternata*, *M. phaseolina* and *R. solani*	Reduction in mycelial growth	[51]
Chitosan	Antifungal	*Alternaria kikuchiana* Tanaka and *Physalospora piricola*	Inhibition of spore germination, germ tube elongation, and mycelial growth	[48]
Rhodamine-labeled chitosan	Antifungal	*F. oxysporum*	Inhibition of mycelial growth, cell membrane permealization, and lysis	[49]
Chitosan NPs	Antifungal and plant growth promotion	*Penicillium expansum*	Reduction in natural and artificial infections. Induction of defense-related genes and resistance in plants	[50]
Chitosan NPs	Antifungal and plant growth promotion	*Sclerospora graminicola*	Promote seed germination and seedling vigor, induced systemic and durable resistance	[66]
Ag-chitosan nanocomposites	Antifungal	Seed-borne fungi	Inhibition of mycelial growth	[67]
Cu-chitosan nanocomposites	Antifungal	*Fusarium graminearum*	Inhibition of mycelial growth	[68]
Chitosan nanocomposites	Antibacterial	*Ralstonia solanacearum*	Inhibition zone production, cell wall modification, loss of flagella and cell lysis	[55]
Chitosan nanocomposites with thyme critical oil	Antibacterial	*Pectobacterium carotovorum*	Inhibition zone production	[57]
Cs/TiO2NPs	Antibacterial	*X. oryzae* pv. *oryzae*	Reduction in infection	[60]

Chitosan nanomaterial	Application	Target pathogen	Effect	Reference
Oligochitosan	Induction of resistance in plants against TMV	*Tobacco Mosaic Virus*	Increase in the levels of intracellular H_2O_2, NO, and phenylalanine ammonia-lyase (PAL)	[65]

Table 1.
Antimicrobial properties of chitosan-based nanomaterials.

an increase in the levels of intracellular H_2O_2, NO, and increased activity of phenylalanine ammonia-lyase (PAL) indicating induction of plant defense response against TMV (**Table 1**).

6. Mode of action of chitosan nanoparticles

6.1 Direct activity against pathogen

ChNPs can directly interact with the cellular membrane of microorganisms due to their unique physicochemical properties and can easily permeate into the cytoplasm. The direct mode of action of ChNPs against fungi includes inhibition of spore germination, germ tube elongation, mycelial growth, and cell lysis. Benhamou [69] conducted ultrastructural studies and reported that chitosan induces numerous structural and morphological changes leading to distorted hyphae. This can be explained as the chitosan particles are polycationic in nature, it allows alteration in membrane permeability and cytoplasmic aggregations. As a result, the activity of enzymes involved in the synthesis and assembly of cell wall polymers are dwindled. The antibacterial effect includes disruption of the bacterial cell wall, cellular membrane, loss of external appendages, such as flagella, finally, leading to cell lysis. None of the studies have proved ChNPs to inactivate viruses and viroids. Most of the studies reported inhibition of virus replication, multiplication, and spread by chitosan. However, against pests and pathogens, ChNPs operate via an indirect mechanism, such as induction of host resistance.

6.2 Indirect mechanism

Chitosan molecule is generally used as an elicitor rather than an antimicrobial agent in plant disease control. It can be recognized by the plant PRRs and can trigger a cascade of defense responses. Chitosan molecule behaves as MAMP/PAMP or general elicitor and induces nonhost resistance in plants along with priming systemic immunity [70]. The cascade of biochemical and molecular reactions induced by chitosan includes enhanced H^+ and Ca^{2+} influx into the cytosol, callose apposition, activation of MAP-kinases, hypersensitive response, oxidative burst, synthesis of phytohormones *viz*., jasmonates and abscisic acid, as well as phytoalexins and PR-proteins [71].

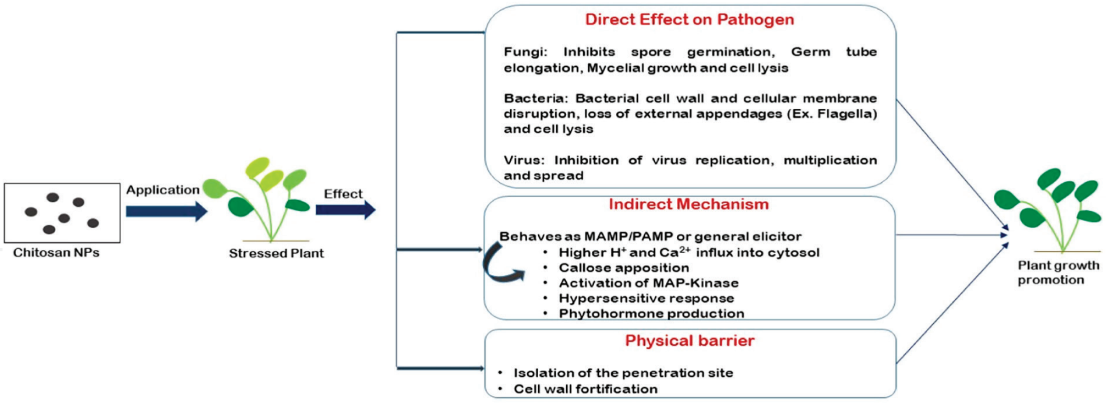

Figure 2.
Schematic representation of the mechanism of action of chitosan nanoparticles on plants.

6.3 Physical barrier in pathogen penetration

ChNPs can agglutinate around the penetration sites of the pathogen after its application on plant tissues and has two major effects. The first effect includes isolation of the penetration site from healthy tissues by forming a physical barrier that prevents further spread of the pathogen. Around the isolated zone, several biochemical changes occur that lead to the elicitation of hypersensitive response, accumulation of H_2O_2 and other free radicals, which lead to cell wall fortification and induction of systemic acquired resistance. The second effect includes the initiation of wound healing process by binding with various materials (**Figure 2**).

6.4 Plant growth promotion

Nano-chitosan imparts an eustress effect on seedling germination and plant growth parameters, such as plant height, shoot length, root length, and biomass content, which have been confirmed through a series of studies. The study conducted on the effect of nano-chitosan on *Phaseolus vulgaris* L. under salt stress conditions revealed that 0.3% nano-chitosan was the best treatment in terms of germination, growth parameters. Also, significant increase in M.S.I, Chl.a, Chl.b, proline, catalase, carotenoids, and antioxidant enzymes were observed [72].

7. Conclusion and future prospects

Chitosan is a naturally occurring miracle compound having enthralling antimicrobial and eliciting properties. Nano-chitosan is gaining attention nowadays due to its greater efficacy and biosafety. Chitosan nanomaterials can be used in varied ways for plant disease management, thereby preserving crop quality and yield. In recent years, several findings have been gathered indicating nano-chitosan as a potential plant health material. However, more studies need to be channelized to unveil the exact mode of action of nano-chitosan specific to the pathosystem. Incorporation of chitosan nanomaterials into integrated pest management practices by devising suitable incorporation techniques need to be pursued. The biopolymer-based nanomaterials need extensive exploration owing to their multifunctional properties and diverse mechanisms. In the coming years, the use of nano-chitosan for combating biotic and abiotic stresses and transport of agrochemicals would be a promising discipline for utility in sustainable agriculture.

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