

# Chapter 13

## Chitosan-Based Nanostructures in Plant Protection Applications



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### 13.1 Introduction

Chitosan is a nontoxic and biodegradable natural biopolymer resulting from the deacetylation of chitin. Chitosan is a linear semicrystalline polysaccharide obtained by deacetylation of chitin and composed by N-acetyl D-glucosamine and D-glucosamine units, linked through  $\beta$  (1 $\rightarrow$ 4) glycosidic bonds, Chitin or poly( $\beta$ -(1 $\rightarrow$ 4)-N-acetyl-D-glucosamine) is prepared by some living organisms, being the structural component of the shells of crustaceans, cell walls of fungi, and exoskeletons of insects (Alves and Mano 2008; Rinaudo 2006; Mano 2008). Most of the naturally occurring polysaccharides are acidic in nature, but chitosan is one of the naturally occurring basic polymers (Prasad et al. 2017a). Chitosan can be dissolved in water under acidic conditions after the amino protonation to discuss positive charges, gelations, and membrane-forming properties (Berscht et al. 1994).

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The physical and chemical properties of chitosan depend mainly on its molecular weight and degree of deacetylation (Shukla et al. 2013). Based on molecular weight chitosan can be classified into three types including; low molecular weight (LMWC), medium molecular weight (MMWC), and high molecular weight (HMWC). Some studies indicated that chitosan with shorter oligomers, such as LMWC, has greater antifungal activity (Rhoades and Roller 2000; Park et al. 2008). Chitosan-based nanoparticles (CHT NPs) can be easily prepared through self-aggregation. The application of nanotechnology is less discovered in agriculture in general, but some substantial research has been done in crop protection (Park et al. 2006; Jo et al. 2009; Nair et al. 2010; Sharon et al. 2010; Ghormade et al. 2011; He et al. 2011; Lamsal et al. 2011; Kim et al. 2012; Perez-de-Luque et al. 2012; Jayaseelan et al. 2013; Wani and Ahmad 2013; Saharan et al. 2013; Prasad 2014; Saharan et al. 2015; Wang et al. 2016; Saharan et al. 2016; Abd-Elsalam et al. 2017; Rubina et al. 2017; Prasad et al. 2014, 2017a; Sangeetha et al. 2017). Chitosan has garnered extensive interest because of its antimicrobial and antifungal properties (Kendra and Hadwiger 1984; Aziz et al. 2015, 2016). In future, there is a urgently need to discover chitosan biopolymer usage not only for its innovative antimicrobial agent but also for its plant defense supporter property to combat future challenges of mutating plant pathogenic population under climate change (Saharan et al. 2014; Prasad et al. 2017b). This is because nanochitosan as compared to bulk chitosan has superior physico-chemical characteristics that provide enhanced biological activities (Saharan et al. 2013; Van et al. 2013). This fact has promoted the study of CHT NPs at using them as new antimicrobial agents (Qi et al. 2004; Wazed et al. 2011). Copper, zinc, and other metal hybrids with chitosan are active components of many agrochemicals like fertilizers and pesticides and serve as cofactors of several enzymes in plant. Thus, Cu and Zn ultimately lead to higher plant growth and contribute in plant disease control (Gornik et al. 2008; Cabrera et al. 2013; Saharan et al. 2016; Rubina et al. 2017). Chitosan-based nanomaterials hold enormous promise with regard to their application in plant protection and growth (Shukla et al. 2013). Although, specific reports has been introduced to evaluate chitosan biopolymer/nanoparticle for use in plant growth and protection by using nanotechnology tools (Shukla et al. 2013; Saharan et al. 2014; Abd-Elsalam et al. 2017; Choudhary 2017; Prasad et al. 2017b). Some pioneering uses of CHT polymer include synthesis of CHT NPs as a valuable delivery system for fertilizers, herbicides, pesticides, and micronutrients for crop growth promotion by a balanced and sustained nutrition (Siddiqui et al. 2015). In addition, CHT NPs can safely deliver genetic material for plant transformation.

## 13.2 Synthesis Chitosan Nanomaterials

### 13.2.1 *Synthesis Chitosan Nanoparticles*

However, several methods have been used to produce chitosan nanoparticulate. Choice of method depends on the shape and particle size requirements (Agnihotri et al. 2004). Chitosan is a hydrophilic polymer with positive charge that comes from

**Table 13.1** Synthesis of chitosan-based nanoparticles: advantages and disadvantages

Methods	Advantages	Disadvantages
Emulsion cross-linking	1. Easy control of particle size	1. Need to remove oil and surfactant
	2. Good loading efficiency	2. Cross-linker can react with active ingredient
	3. Good stability of nanomaterials	3. Purification of nanoparticle is tedious process
Emulsion-droplet coalescence method	1. Higher encapsulation efficiency	1. Excess alkali induces more precipitation and leads to increased particle size
	2. No cross-linker leads to higher zeta potential	2. Due to absence of cross-linker nanoparticles stability decreases
	3. No reactivity of cross-linker to active ingredient	
Ionic gelation method	1. Reduced the chemical side effect	1. Partial size, distribution, and stability strongly affected by degree of deacetylation, MW of chitosan and molar ratio of chitosan and TPP
	2. Better control on physicochemical feature of nanoparticles	
	3. Easy and fast	
Reverse Micellar Method	1. Stable and smaller and monodispersed nanoparticles with suitable polydispersity index	1. Cumbersome procedure
		2. Chance of side effect of reaction components (solvent, surfactant, etc.)
Sieving method	1. Very simple and rapid procedure	1. Need specialized sieve with particular size for desirable size of nanoparticles
	2. Easy mass synthesis	
Spray drying method	1. Mechanized method for mass production	1. Size of nanoparticles depends on nozzle size, flow rate, and temperature of air
		2. Temperature-sensitive component could not be encapsulated

Agnihotri et al. (2004), Kashyap et al. (2015)

weak basic groups, which provides it specific characteristics from the technological point of view (Lopez-Leon et al. 2005). Recently, chitosan nanoparticles have received great attention in numerous fields because of their physicochemical and biological properties (Racovita et al. 2008; Shi et al. 2011). Some advantages and disadvantages of the methods used for synthesis of nanochitosan are summarized in Table 13.1.

### 13.2.1.1 Emulsion Cross-Linking Method

This method utilizes the reactive functional amine group of chitosan cross links with aldehyde groups of the cross-linking agent. Actually, chitosan solution is emulsified in oil phase (water-in-oil emulsion) and the aqueous droplets are stabilized using an appropriate surfactant. The stable emulsion was reacted with an appropriate

cross-linking agent such as glutaraldehyde to stabilize the chitosan droplets. The nanoparticles are then washed and dried (Agnihotri et al. 2004).

### **13.2.1.2 Ionotropic Gelation Method**

Ionotropic gelation method is the modified method linking multiphase for the production of biopolymeric oil-core microcapsules due to its nontoxic and slight processing conditions. This technique is based on the electrostatic interactions between the chitosan amine group and a polyanion such as tripolyphosphate. In this method, chitosan is dissolved in water or in weak acidic medium. This solution is then added dropwise under continuous stirring to the solutions containing other counterions. Due to the complexation between oppositely charged species, chitosan goes through ionic gelation and precipitate to produce spherical CHT NPs (Racovita et al. 2008).

### **13.2.1.3 In Coacervation/Precipitation Method**

In the coacervation/precipitation method, chitosan solution is sprayed into sodium hydroxide, NaOH methanol, or ethanediamine alkaline solutions using compressed air, which in turn creates coacervated chitosan droplets in the form of nanoparticles (Shi et al. 2011). Emulsion–bead combination strategy incorporates both emulsion cross-connecting and precipitation. A stable emulsion containing the aqueous chitosan solution in oil and a second emulsion containing an NaOH solution are formed. By mixing the two emulsions under high magnetic stir, droplets of each emulsion collide at random, coalesce, and lastly precipitate as small size particles (Shikata et al. 2002).

### **13.2.1.4 In the Reverse Micelles Technique**

In the reverse micelles technique, a surfactant is dissolved in organic solvent to formulate reverse micelles. The aqueous phase containing the chitosan is mixed to this emulsion with constant vortexing and the chitosan nanoparticles forms in the core of the reverse micelles (Agnihotri et al. 2004).

### **13.2.1.5 Self-Assembly**

Self-assembly strategy depends on cationic and hydrophobic properties of chitosan, cationic chitosan derivatives can be simply adsorbed onto the colloid surface of anionic inorganic (bentonite) suspensions because of electrostatic attraction. This strategy is described by dispersion took after by particular relationship of atoms through noncovalent interactions, including electrostatic as well as hydrophobic connections (Ichikawa et al. 2005).

### ***13.2.2 Synthesis Chitosan Nanocomposites***

Chitosan nanocomposite generally refers to chitosan polymer containing dispersed nanofillers having an average particles size less than 100 nm. Thus this composite retains exceptionally enhanced properties pertaining to both of polymer and nanoparticles. By and large, regular polymers are considered as great facilitating materials for nanoparticles especially for natural applications, because of their maintainability, eco-accommodating property, nontoxicity, biodegradability, and biocompatibility. The natural inorganic half-breed nanocomposites have been seen to be valuable in numerous fields like medication conveyance, tissue building, bundling material, covering, and sensors (Rhim et al. 2006; Di Carlo et al. 2012). The primary methods that have been utilized to deliver chitosan nanocomposites, in particular dissolvable throwing are solidify drying, layer-by-layer, and electrospinning (Moura et al. 2016).

#### **13.2.2.1 Solvent Casting**

Dissolvable throwing is a standout amongst the most widely recognized strategies for planning of CHI nanocomposites films and membranes (Caridade et al. 2013; Zheng et al. 2015). The polymer is broken down into a dissolvable and then cast onto a surface, for example, glass Petri dish. The dissolvable is accordingly permitted to dissipate at room temperature or in air stove, and from that point onward, the films/membranes are separated (Ambrosio 2009).

#### **13.2.2.2 Freeze Drying**

Stop drying method was utilized for the arrangement of exceedingly porous scaffolds by inducing thermal phase separation. Usually, the solution temperature is brought down until solid–fluid demixing happens, forming two unique stages: solidified dissolvable and polymer stage. At this point, the solidified dissolvable, through sublimation, leaves the polymeric structure framing a pore. The resultant structure can be controlled by changing the kind of polymer and its concentration (Sarmiento and Neves 2012).

#### **13.2.2.3 Layer-by-Layer Assembly**

Layer-by-layer (LbL) meeting, proposed by way of Iler in 1966, is a technique able to enhance surfaces and fabricate especially ordered polymeric films and nanocomposites over extraordinary varieties of substrates (Decher 1997; Borges et al. 2014). The current technique is based on the sequential adsorption of different macromolecular components, which are attracted to each other due to electrostatic interactions, hydrogen bonding, van der Waals forces, and electron exchange, among others (Borges et al.

2014; Borges and Mano 2014). Various LbL tactics can be used to build up a multi-layer film, including dip coating, spin coating, and spraying coating (Richardson et al. 2015). Due to its versatility and notable availability of building blocks (e.g., CNTs, clays, NPs, polymers), this technology permits fabrication of multilayered gadgets of any nature, length, shape, and chemical composition, assuring the improvement of nanostructures with favored geometries and functionalities (Costa and Mano 2014). Furthermore, the properties of multilayered devices may be tuned through solution pH, temperature, or ionic electricity (Borges and Mano 2014).

#### 13.2.2.4 Electrospinning

Electrospinning represents an appropriate technique to produce fibers with diameters in the nm– $\mu$ m period scale, since it permits morphology, porosity, and composition to be managed by the use of relatively unsophisticated equipment. Typically, the electrospinning process uses an electric field created between the polymer solution and the collector, which generates internal repulsive forces in the polymer solution and, at a critical point, causes the expulsion of the polymer solution in shape of fibers towards the collector (Martins et al. 2008; Teo and Ramakrishna 2006). There are three special kinds of electrospinning: wet–dry electrospinning, moist–wet electrospinning, and coaxial electrospinning. The main distinction between the first two techniques is that the moist–dry approach makes use of a risky solvent that evaporates because the fibers are spun via the collector, while the moist–wet approach spins a nonunstable solvent to a collector with a 2D solvent. Regarding the final technique, it is possible to achieve fibers with a center-sheath shape, as two exclusive components may be spun at the same time (Zheng et al. 2015).

#### 13.2.2.5 Template Polymerization

In the present method, chitosan is firstly dissolved in an acrylic monomer solution below magnetic stirring. Due to the electrostatic interplay, the negatively charged acrylic monomers align along the chitosan molecules. After whole dissolution of chitosan, the polymerization is commenced through adding potassium persulfate initiator (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) underneath stirring at 70 °C. The entire polymerization ends in the appearance of an opalescent answer, indicating the nanoparticles formation (Fang et al. 2009; Shi et al. 2011).

### 13.3 Nanochitosan Characterization

#### 13.3.1 X-Ray Powder Diffraction

X-ray Powder Diffraction (XRPD) analysis was performed to confirm the crystalline structures of magnetite present in chitosan/Fe<sub>3</sub>O<sub>4</sub> nanocomposites. The samples were analyzed by X-ray powder diffractometer Xpert Pro MPD (Panalytical) using

Bragg–Brentano geometry in the range  $15\text{--}70^\circ$  with a rate of  $1^\circ \text{ min}^{-1}$ . CuK $\alpha$  radiation ( $k = 1.54059 \text{ \AA}$ ) was used and the tube operated at 40 kV and 30 mA (Freire et al. 2016).

### ***13.3.2 Fourier Transform Infrared Spectroscopy***

Fourier Transform Infrared Spectroscopy (FTIR) evaluation was carried out in a PerkinElmer 2000 spectrophotometer used to report spectra within the range between 4000 and  $400 \text{ cm}^{-1}$ . In previous measurements, the samples were dried and level-headed to be powdered and pressed ( $\sim 10 \text{ mg}$  of sample to  $100 \text{ mg}$  of KBr) in disk format (Freire et al. 2016).

### ***13.3.3 Transmission Electron Microscopy (TEM)***

In Transmission Electron Microscopy (TEM), the snap shots were recorded through a JEOL JEM-1400 electron microscope operating at an accelerating voltage of 120 kV. The samples had been prepared by way of diluting nanoparticles dispersion in distilled water. Then, one droplet of the sample was placed on 300-mesh carbon-covered copper grids and dried overnight under ambient conditions. The size distribution was decided by means of 50 randomly selected debris in exclusive regions of the increasing TEM micrograph (Freire et al. 2016).

### ***13.3.4 Thermogravimetric Analysis***

Thermogravimetric analysis (TGA) is a useful method for examining thermal stability of materials as well as for compositional information. Five milligram of nanoparticles were executed in nitrogen atmosphere by employing a Thermogravimetric Analyzer Q50 V20. The lack of mass was monitored heating up samples from  $25$  to  $900^\circ \text{C}$  in a fee of  $10^\circ \text{C min}^{-1}$ . The zero time for the thermal degradation observe became taken after temperature stabilization (Freire et al. 2016).

### ***13.3.5 Dynamic Light Scattering***

Dynamic light scattering (DLS) was used for the size of particle length, polydispersity index (PDI), and zeta capacity of NPs via Zetasizer model PSS0012–22 (Malvern, UK). The evaluation was completed at a scattering angle of  $90^\circ$  at RT. Sample was accurately diluted with distilled water prior to size (Kheiri et al. 2016).

## 13.4 Application of Nanochitosan in Plant Protection

Natural defense reaction of plants against pathogenesis depends upon early detection of pathogens. In fact, during the process of evolution, plant researchers have evolved numerous techniques to combat some plant pathogens. This induction of herbal protection response consists of over expression of various defense-associated genes and enzymes, increased accumulation of phenolic compounds, cellular wall synthesis, etc. (Sánchez et al. 2009; Chandra et al. 2015). Recently, chitosan nanoparticles have shown significant antimicrobial activity against fungal plant pathogens (Saharan et al. 2013). Chitosan nanoparticles serve as the precise choice for the control of pests which might eliminate the toxic pesticide use and additionally enhances the yield of soybean (Kendra and Hadwiger 1984). Application of chitosan-based nanostructures in plant protection is shown in Fig. 13.1 and Table 13.2.

### 13.4.1 Antimicrobial

Chitosan nanoparticles specific extra affinity toward pathogen's outer membrane and therefore without difficulty enter into the pathogens' cell (Van et al. 2013). Several research confirmed that chitosan isn't best an antimicrobial agent however additionally an effective elicitor of plant systemic received resistance to pathogens (Sharp 2013; Katiyar et al. 2015; Xing et al. 2014), enhancer and regulator of plant growth, development and increase crop yield (Gornik et al. 2008; Cabrera et al.



Fig. 13.1 The potential application of nanochitosan in plant protection



**Table 13.2** Chitosan-based nanomaterials used in plant growth and crop protection

Chitosan NMs	Applications	Findings	References
Chitosan NPs	Antifungal activity	Significantly delayed mycelia growth of <i>Rhizopus</i> spp., <i>Colletotrichum</i> spp. ( <i>C. capsici</i> , <i>C. gloeosporoides</i> ) and <i>Aspergillus niger</i>	Chookhongkha et al. (2012)
	Antifungal activity	Effective against <i>A. alternata</i> , <i>M. phaseolina</i> and <i>R. solani</i>	Saharan et al. (2013)
	Plant promotion	Study on chitosan nanoparticles on biophysical characteristic and growth of Robusta coffee in green house	Van et al. (2013)
	Controlled delivery system/bioactivity in plants	Effective delivery system for sustainable agriculture	Kashyap et al. (2015)
	Defense activity	Positive modulator of innate immune responses in plants	Chandra et al. (2015)
	Defense activity	biological pesticide for controlling Fusarium head blight	Kheiri et al. (2016)
	Plant promotions Antifungal activity	Chitosan nanoparticle induced defense responses in Finger millet plants against blast disease caused by <i>Pyricularia grisea</i>	Manikandan and Sathiyabama (2016)
	Plant promotions Antifungal activity	promote seed germination and seedling vigor, induced systemic and durable resistance against <i>Sclerospora graminicola</i>	Siddaiah et al. (2018)
Ag-Chitosan NCs	Antifungal activity	Seed-borne fungi	Kaur et al. (2012)
Cu-Chitosan NCs	Antifungal activity	<i>Fusarium graminearum</i>	Brunel et al. (2013)
	Seedling growth promotions, Antifungal activity	Efficacy against <i>Alternaria solani</i> and <i>Fusarium oxysporum</i>	Saharan et al. (2015)
	Seedling growth promotions, Antifungal activity	<i>Rhizoctonia solani</i>	Abd-Elsalam et al. (2017)
	Growth promotory effect on maize seedling growth Antifungal activity	<i>Fusarium verticillioids</i>	Choudhary (2017)
	Antifungal activity	Sclerotia-forming plant pathogenic fungi	Rubina et al. (2017)
Chitosan and methyljasmonate	Defense activity	Efficacy against <i>Alternaria alternata</i> and enhancing activity of cherry tomato fruit defense mechanisms	Chen et al. (2014)

(continued)

**Table 13.2** (continued)

Chitosan NMs	Applications	Findings	References
Chitosan/boehmite	Antifungal activity	<i>Monilinia laxa</i>	Cindi et al. (2015)
Cu-chitosan Hydrogel	Plant growth promoting	Positive effects on tomato growth	Juárez-Maldonado et al. (2016)
Oleoyl-chitosan nanoparticle	Antifungal activity	Synthesis and in vitro antifungal efficacy of oleoyl-chitosan nanoparticles against plantpathogenic fungi	Xing et al. (2016)

2013; Wang et al. 2016). Chitosan NPs treatment of leaves and seeds produced significant improvement in the plant growth and innate immune response through induction of defense enzyme activity, upregulation of defence related genes including that of several antioxidant enzymes as well as elevation of the levels of total phenolics (Chen et al. 2014; Chandra et al. 2015).

However, very few reports have been conducted on the biological activity of chitosan nanoparticles to control of plant pathogenic bacteria. An in vitro observe in which chitosan nanoparticles and chitosan nanocomposites with lime essential oil and thyme critical oil was accomplished. The effects indicated an inhibition of the *Pectobacterium carotovorum* via the treatment the usage of chitosan nanocomposites with thyme essential oil (Sotelo-Boyás et al. 2016). Concentration 1000 ppm of Cu-chitosan NPs have antibacterial effect against *Pseudomonas syringae* pv. *glycinea* that cause bacterial blight of soybean (Swati et al. 2017).

Chitosan was proven to inhibit the systemic propagation of viruses and viroids throughout the plant and to enhance the host's hypersensitive response to infection (Pospieszny et al. 1991; Faoro et al. 2001; Chirkov 2002). The degree of suppression of viral infections diverse based on the type of chitosan molecular weight (Kulikov et al. 2006). However, not one of the studies that investigated this effect has in reality proven the capacity of chitosan in absolutely inactivating viruses or viroids. Most literature i.e, (Kulikov et al. 2006) Reported on the inactivation of replication, which cause the stoppage of multiplication and spread. This will be linked to the reality that upon penetration into plant tissues, chitosan nanoparticles tightly bind nucleic acids and purpose a ramification of damages and selective inhibitions. Diverse time requirements by means of chitosan remedies for improvement of most appropriate defense stimulation ability was recorded in advance research in specific plant-pathogen systems wherein tobacco-TMV interactions (Zhao et al. 2007).

### 13.4.2 Antifungal

Chitosan NP were stated to have antifungal activity against specific plant pathogens. Nanoparticles on their own can negotiate mobile partitions and membranes at some distance more effectively than compared to the middle molecules they are organized

from. This partially explains why chitosan NPs were discovered to demonstrate higher immune stimulation in comparison to chitosan itself (Chandra et al. 2015). Chitosan can effectively inhibit the development of phytopathogenic fungi at different life cycle ranges. For instance, chitosan completely inhibited spore germination, germ tube elongation, and mycelial growth of *Alternaria kikuchiana* Tanaka and *Physalospora piricola* Nose at 5 g/L in vitro assay (Meng et al. 2010). In the pear fruit, treatments with chitosan reduced the ailment prevalence and inhibited the lesion growth as a result of the two aforementioned phytopathogenic fungi (Meng et al. 2010). The antifungal effect of rhodamine-labeled chitosan on *Fusarium oxysporum* was greater than on *Pochonia chlamydosporia*. Chitosan penetrated cell membranes of *F. oxysporum* by plasma membrane permeabilization, resulting in cell lysis (Palma-Guerrero et al. 2010). In commercial wine grapes, chitosan efficaciously inhibited growth of *Botrytis cinerea* in liquid and suppressed gray mold on detached grapevine leaves and bunch rot (Reglinski et al. 2010). The chitosan TPP NPs with sizes between 80 nm and 20 nm were evaluated for their antifungal efficacy against *Aspergillus parasiticus*, and an improved antifungal potential was found in comparison to the chitosan solution (Cota-Arriola et al. 2013). The antifungal activity of chitosan nanoparticles in controlling both *A. niger* and *F. solani* was recorded (Ing et al. 2012). Chitosan–silver nanoparticles were investigated for control of seed-borne pathogens in chickpea. Significant antifungal activity against *Aspergillus flavus*, *R. solani*, and *A. alternata* was evaluated (Kaur et al. 2012). Chitosan has strong antifungal activity against *Rhizoctonia solani* in rice. Two kinds of acid-soluble chitosan (with different degrees of deacetylation) caused a 60–91% inhibition in mycelial growth, 31–84% inhibition of disease incidence, and 66–91% inhibition in lesion length (Liu et al. 2012). The antifungal impact of chitosan on fungal growth of *Rhizopus* sp., *C. capsici*, *C. gloeosporioides*, and *A. niger* in seeds of chilli pepper (*Capsicum* sp.) was confirmed, showing at the least mycelial increase of 2.8, 2.2, 2.4, and 5.5 mm, respectively (Chookhongkha et al. 2013).

The antifungal properties of chitosan nanoparticles with silver functionalized with 4(E)-2-(3-hydroxynaphthalene-2-yl) diazen-1-yl and benzoic acid for controlling *A. flavus* and *Aspergillus terreus* was evaluated. The fungal increase inhibition was proven between 20.2 and 27.0 mm, respectively (Mathew and Kuriakose 2013). Chitosan nanoparticles displayed robust inhibition of mycelial increase of *M. phaseolina*. A maximum 87.6% inhibition degree was recorded at 0.1% of chitosan nanoparticles, so specifically chitosan and Cu-chitosan nanoparticles proved their uniform size and stability which may additionally make a contribution to their higher antifungal efficacy against both of *A. Alternata*, *M. phaseolina* and *R. solani* in vitro assay. Cu-chitosan nanoparticles also confirmed maximum inhibition rate of spore germination of *A. alternata* (Saharan et al. 2013). Chitosan nanoemulsion was used for controlling *C. gloeosporioides* in vitro; the effects showed that the low molecular weight chitosan at a concentration of 1.0% had the best results in phrases of inhibition of conidial germination of the fungus (Zahid et al. 2013). The effect of peppermint vital oil (*Mentha piperita*) encapsulated in chitosan nanogels with cinnamic acid on *A. flavus* was studied. Inhibitory effect at the mycelial growth of the fungus at a concentration of 800 mg/mL was observed in vitro (Beyki et al. 2014). The antifungal impact of chitosan combined with silver for controlling *C. gloespo-*



**Fig. 13.2** Effect of different treatments on disease severity of FHB, 4 weeks after fungus inoculation. (a) Control (distilled water), (b) CS/NPs, (c) Control (0.5% v/v acetic acid aqueous solution), (d) CS, (e) Positive control (Tilt fungicide). (Reprinted from Kheiri et al. 2016)

*rioides* was evaluated using mango fruit, the varied concentrations was decreased the anthracnose by means of 45.7 and 71.3%, respectively (Chowdappa et al. 2014).

The polyethylene terephthalate punnets containing thyme oil and wrapped with chitosan/boehmite nanocomposite lidding films drastically reduced the incidence and severity of brown rot because of *Monilinia laxa* in artificially inoculated peach fruit (cv. Kakawa) held at 25 °C for 5 days and considerably reduced brown rot occurrence additionally at decrease temperatures (Cindi et al. 2015). Encapsulation of thyme EO in chitosan–benzoic acid nanogel has greater antimicrobial property in *A. flavus* for the protection of tomatoes (Khalili et al. 2015). As a broad-spectrum fungicide, primarily chitosan-based nanoparticles have been showed to be fungicidal against many pathogenic fungi. Cu–chitosan nanoparticles at 0.12% concentration caused 70.5 and 73.5% inhibition of mycelia growth in *Alternaria solani* and *Fusarium oxysporum*, respectively (Saharan et al. 2015). The management of Fusarium head blight (FHB) by using chitosan (CS) and chitosan nanoparticles (CS/NPs) has been studied. CS/NPs prevented fungal growth and CS/NPs may be a beneficial organic pesticide for controlling FHB. Spikelets treated with CS and CS/NPs showed gradual infection (Kheiri et al. 2016). The results confirmed that the percentage of disease severity reduced when CS and CS/NPs were employed before the fungus inoculation on the host (Fig. 13.2). Thus, CS and specifically CS/NPs, synthesized via an appropriate technique, can be used as biological pesticide in controlling fungal plant pathogens. Mycelial growth showed that there were considerable differences in tolerance to primarily chitosan-based nanoparticles among the various fungi examined. Plant pathogenic fungi including; *N. sphaerica*, *B. dothidea*, *N. oryzae*, and *A. tenuissima* was sensitive to O-chitosan nanoparticles, whilst *G. zeae* and *F. culmorum* was resistant. The anti-

fungus activity of nanoparticles on four chitosan-sensitive fungi was concentration-dependent. The maximum value of antifungal index was discovered in medium containing nanoparticles at 2.0 mg/mL for each fungus (Xing et al. 2016). Synthesize and describe Cu-chitosan NCPs and evaluates its antifungal activities against *Fusarium verticillioids* causing publish flowering stalk rot (PFSR) disorder of maize. All collectively Cu-chitosan NCs has exquisite potential as antifungal agent against PFSR of maize in pot condition as well as in field condition (Choudhary 2017). The antifungal activity of  $\alpha$ -chitin nanoformulations with sizes ranging from 80–100 nm diameters became evaluated on *A. niger* fungal increase, and they showed 87% of fungal growth inhibition (Salaberria et al. 2017).

Chitosan NPs have been synthesized from low-molecular-weight chitosan having better degree of acetylation was evaluated for thier efficacy aganist downy mildew disease of pearl millet resulting from *Sclerospora graminicola* (Siddaiah et al. 2018). This report confirmed that pearl millet seed remedy with nanochitosan particles was appreciably promoted seed germination and seedling vigor and, in addition, effectively induced systemic and durable resistance against downy mildew disease under greenhouse conditions. Nanochitosan has a broad-spectrum activity and an extensive antifungal activity on plant pathogenic fungi (Table 13.2).

### 13.4.3 Postharvest

Plant pathogenic bacteria negatively affect a wide variety of vital fruit and vegetables during the growing season and the duration of postharvest storage (Li et al. 2009a, b, 2010).

Traditional antimicrobials have been extensively used for decades. Recently, chitosan coating has emerged as a super alternative to chemically synthesized pesticides. In postharvest production, to prevent unwanted losses, chitosan biopolymer has emerged as a promising coating agent for end-product and vegetables due to its antimicrobial and defense eliciting properties. Some studies confirm that the in vitro growth of *Pseudomonas fluorescens* causing bacterial head rot of Broccoli (Li et al. 2009a, b), and *Burkholderia seminalis* inflicting bacterial fruit rot of apricot (Lou et al. 2011), have been markedly inhibited by chitosan under various environmental conditions. In addition, the antibacterial agent comprising 1–2%, also chitosan has accurate results in inhibiting the bacteria in the fruit and vegetable in reserving process and has inhibiting effect to the rot of fruit and vegetable. Besides, it has simple processing technology and low fee and is in accordance with the developmental path of modern antibacterial agent (Feng et al. 2009). The most efficient treatment to reduce disease occurrence has been the ones based on *Cinnamomum zeylanicum* essential oil (CEO)-loaded chitosan NPs. This treatment become not simplest powerful in controlling cucumber decay, however additionally in delaying disease symptoms and slowing down *P. drechsleri* increase during the storage stage. Uncoated cucumbers began to decay from the fourth day of storage (Mohammadi et al. 2015). Chitosan can also help to preserve the safety of fit to be eaten products.

The protection of fresh cut broccoli against *E. coli* and *Listeria monocytogenes* was confirmed with the aid of chitosan alongside bioactive components which includes bee pollen and extracts from propolis and pomegranate. Coating of chitosan on fruits and greens induces various defense enzymes, together with phenylalanine ammonia lyase (PAL), chitinase, and glucanase. Introduction of chitosan nanofilm composites made from chitosan and other antimicrobial agents could be more powerful in controlling postharvest losses of end-product and vegetables.

#### **13.4.4 Food Preservation**

Food packaging nanoparticles with antimicrobial interest which include chitosan (Qi et al. 2004; Tan et al. 2013), chitosan nanoparticles, and different inorganics. Combination of silver nanoparticles to the polymer matrix made the nanocomposites more appealing to be used in packaging (Duncan 2011; Fernández-Saiz and Lagaron 2011; Molina and Mejía 2016). Incorporation of those substances into the polymer matrix renders it lighter, stronger, fire resistance, better thermal properties, much less permeable to gases. Development of nanocomposites (up to 5% w/w nanoparticles) has been reported (Llorens et al. 2012). Chitosan nanoparticles (89 nm) and pectin (used as plasticizer) have been added into banana puree films (Martelli et al. 2013). The chitosan derivative coating was effective in reducing the decay of green asparagus caused by *F. concentricum*. In vitro assay, L-chitosan and H-chitosan inhibited the radial growth of *F. concentricum*, with an extremely good impact at concentration of 4 mg/ml, and totally inhibited spore germination at a concentration of 0.05 mg/ml, indicating that chitosan derivatives have been either fungistatic or fungicidal. The found inhibition of degradation and augmented lignification became attributed to the fungistatic activity of chitosan and its ability to induce a protection response (Qiu et al. 2014). The use of nanoparticles might assist in manufacturing of recent meals packaging materials with advanced mechanical, barrier and antimicrobial properties to growth shelf life (Chaudhry et al. 2008; Mihindukulasuriya and Lim 2014). Incorporated ZnO nanomaterial at diverse concentrations in a chitosan polymer with neem critical oil to enhance the properties of the bionanocomposite films (Sanuja and Agalya 2015).

#### **13.4.5 Antimicrobial Mechanism**

Chitosan has been validated to be a natural molecule that induces numerous organic responses in plant life, dependent on its structure, concentration, and on species and developmental stage of the plant (Malerba and Cerana 2016). Chitosan NPs remedy of leaves and seeds produced giant development inside the plant increase and innate immune reaction via induction of defense enzyme interest, up-regulation of defense-associated genes together with that of several antioxidant enzymes as well as

elevation of total phenolics (Chen et al. 2014; Chandra et al. 2015). Natural protection response of plant life depends upon early detection of pathogens during the pathogenesis. Chitosan, whilst realised to plant tissues, often agglutinate across the penetration sites and has two main effects. The first one is the isolation of the penetration plant host via the formation of a physical barrier stopping the pathogen from spreading and invading other wholesome tissues. This phenomenon resembles the abscission zones regularly found on leaves stopping several necrotrophic pathogens from spreading further. It is widely found on potato tubers for instance (El Hadrami et al. 2009).

### 13.4.6 Induce Resistance

Furthermore, El Hassni et al. (2004) observed the impact of chitosan in date palm in reaction to *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of a fusarium wilt on this crop. Beside an instantaneous toxicity of the molecule on the fungus, the authors confirmed an enhancement of critical additives of the host resistance. When injected into the roots at different concentrations, chitosan elicited date palm peroxidase and polyphenoloxidase activities, and increased the extent of phenolic compounds. Among the accrued phenolics, there was growth in content of unique nonconstitutive hydroxycinnamic acid derivatives, regarded to be of great importance in the resistance of this plant to this vascular fusariosis. Similarly, remedy of wheat seeds with chitosan found a growth in hydroxycinnamic (i.e., p-coumaric, caffeic, and ferulic) and benzoic (i.e., benzoic, protocatechuic, and gallic) acid derivatives, key to an increase in lignin synthesis and accumulation (Reddy et al. 1999). Chitosan is thought to behave as powerful inducer, enhancing a battery of plant responses each regionally around the infection sites and systemically to alert healthy elements of the plant. These encompass early signaling events in addition to the buildup of defense-related metabolites and proteins including phytoalexins and PR-proteins (Reddy et al. 1999; El Hadrami et al. 2009; Hammerschmidt 1999; Vander et al. 1998; Wang et al. 2008). Hence, as an exogenous elicitor, chitosan can stimulate resistance in plant host by way of increasing a few defense-related enzymes activities, together with phenylalanine ammonia-lyase (PAL), peroxidase(POD), catalase (CAT), superoxide dismutase (SOD), and polyphenol oxidase (PPO) activity (Xing et al. 2015). Finally, Chandra et al. (2015) have mentioned that accumulation of chitosan NP will increase plant protection through increasing the stages of SOD and CAT. Chitosan NP binds extracellularly across the cellular wall of leaves. One of the most crucial signaling molecules is nitric oxide (NO), which is also related to a variety of physiological procedures including induction of defense mechanism in plants. Plants treated with chitosan NP showed increased degrees of NO, in comparison to nontreated plants with chitosan NP (Raho et al. 2011; Malerba et al. 2012).

Chitosan NP treated sets resulted in up-regulation of PAL leading to a higher level of phenolic compound accumulation. These accumulated compounds help in

alteration to distinct environmental conditions and offer resistance against pathogen. In the presence of NADPH, ANR uses anthocyanidins as substrates to synthesize EC. EC, sooner or later, transforms into proanthocyanidins, which are broadly dispensed as plant defense compounds having great toxicity toward pathogens. High levels of flavonoid accumulation are probably an illustration of superior resistance to flowers. Chitosan NP-treated flora, higher expression of SOD and CAT was observed, resulting in increase of these enzymes. SOD and CAT are the important antioxidant enzymes involved with ROS scavenging (Chandra et al. 2015). Polyphenol oxidase, catalyzing the phenolic materials to provide lignin, is everywhere among angiosperms and said to be involved in plant protection by means of helping the formation of lignin that contributes to the reinforcement of the cell wall structure heading off the penetration of pathogen (Li and Zhu 2013). ROS, Ca<sup>2+</sup>, nitric oxide (NO), ethylene (ET), jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) are all engaged in chitosan-mediated sign pathway (Xing et al. 2015). More details related to antimicrobial mechanism for nanochitosin was reviewed in Chap. 11.

### 13.4.7 Anti-insects

Chitosan has been found to expose robust insecticidal activity in a few plant pests (Zheng et al. 2005; Rabea et al. 2005). Chitosan (i.e., N-alkyl-, N-benzylchitosans) are made to be had through chemical synthesis, their insecticidal activities are being reported using an oral larvae feeding bioassay (Rabea et al. 2005; Badawy et al. 2005). Encapsulated microcrystals of the insecticide imidacloprid (IMI) with the aid of LbL assembly the use of chitosan and sodium alginate accompanied by way of addition of photocatalytic NPs (Guan et al. 2008). The insecticide etofenprox was encapsulated the use of a nanosized chitosan carrier in three types according to a difference in release patterns by adjusting the molecular weight and concentration of chitosan. Release properties of etofenprox and its organic activity against *Spodoptera litura* suggested that such managed-release method is used as a technique for preventing loss of etofenprox, increasing its activity against the target pest (Hwang et al. 2011).

The entomopathogenic fungi *Nomuraea rileyi* were investigated against *S. litura*, and chitosan nanoparticle coated fungal metabolite (CNPCFM) showed better pesticidal activity, as compared with Uncoated Fungal Metabolite (UFM) and Fungal Spores (FS) (Chandra et al. 2013). Chitosan nanoparticles integrated insecticidal protein beauvericin (CSNp-BV) became organized by using ionic gelation technique to enhance insecticidal activity against *S. litura*. Pesticidal interest found out that all lifestyles stages have been susceptible to the CSNp-BV formulation and the maximum mortality was recorded in early larval instars. CSNp-BV treatment reduced pupal and adult emergence (Bharani et al. 2014).

Chitosan (CS)-g-poly (acrylic acid) PAA nanoparticles reduced egg laying of *Aphis gossypii* ( $20.9 \pm 9.1$  and  $28.9 \pm 9.2$  eggs/woman for laboratory and below



semi-discipline situations, respectively) than manage ( $97.3 \pm 4.9$  and  $90.34$ . Nine eggs/female for laboratory and below semi-subject situations, respectively (Sahab et al. 2015). Chitosan nanoparticles decreased egg laying of *Callosobruchus maculatus* ( $10.9 \pm 9.9$  and  $19.9 \pm 9$ . Nine eggs/female laboratory and under semi-storage conditions, respectively (Sahab et al. 2015). Under semi subject situations, the wide variety of *Schistocerca gregaria* had been notably reduced after the chitosan and nanochitosan remedy, the quantity of infestations with *S. gregaria* reduced to  $29 \pm 3.6$  and  $8 \pm 1.1$  individuals after 120 days of remedies (Sabbour 2016). Chapter 1 was tested the application information of nanotechnology for insect's management.

### 13.4.8 Growth Promotions

During the past 100 years, chemical fertilizers and pesticides were used to face these problems and increase yield. The huge utilization of these products raised productivity significantly, but it also led to reduced biological diversity and degraded natural and agricultural systems. In addition, residue accumulation led to environmental pollution and public health problems, with development of resistant pests (Sun et al. 2012). Therefore, alternative techniques that include nanomaterials are important to address the issues of lowering the environmental impact without affecting agricultural productivity and economic viability for farmers (Ghormade et al. 2011; Kah and Hofmann 2014).

Micro-chitosan remedies have plant growth promoting activities, resulting in increased yields and plant health in numerous plants and fruits. The activation of defensive mechanisms in plant tissues with chitosan inhibited the growth of taxonomically unique pathogens (Vasyukova et al. 2001). Therefore, radicles, after penetrating the seed coatings may want to contact the NPs directly. The presence of chitosan NPs in huge quantities on the root floor should adjust the surface chemistry of the root and block the root openings and both hydraulic and nutrient uptake in roots is inhibited. Therefore, plant growth is negatively affected because of NPs (Behboudi et al. 2017). In the case of tomato seeds, Saharan et al. (2016) observed that Cu-chitosan NPs at high concentration showed more inhibitory effect on seedling growth. The seed quality of minitubers derived from chitosan treatments in vitro was improved, giving rise to field plants with increased tuber numbers and yields (Kowalski et al. 2006). Chitosan and chitooligosaccharides are as molecular signals to induce plant promoter and develop disease resistance system in plants (Bueter et al. 2013). Chitosan oligomer has large influences on biophysical feature, growth, and improvement of coffee such as increasing nutrient uptake, content material of chlorophyll and information of growth and yield (Dzung et al. 2011). Chitosan has remarkable film-forming property making it a clean agent to form a semipermeable coat at the seed surface that may keep the seed moisture and take in soil moisture which hence can promote seed germination. Moreover, it restrains the seed respiration through preventing oxygen entry, proscribing loss of  $\text{CO}_2$  and

keeping high concentration of CO<sub>2</sub> inside the film (Furbank et al. 2004). Chitosan also can increase soluble sugar content and improves the activity of protease to increase loose amino acid content (Zeng et al. 2012).

The effect of chitosan NPs on seed germination and seedling vigor has been evaluated in diverse vegetation for promotion of plant growth through increasing the uptake of vitamins and water through adjusting cellular osmotic pressure (Guan et al. 2009; Katiyar et al. 2015). Insufficient studies were carried out on using Cu/Zn chitosan NPs in seed germination and seedling growth. Wheat growth was promoted in terms of germination ability, root period, and seedling top by way of the motion of oligochitosan (Ma et al. 2014). Positive response of micro-chitosan on seed germination and seedling increase results in improvement of nanoformulation of chitosan. Chitosan nanoparticles have nanometer size and they can inter into the cells of the plants simply and improve their better bioactivities (Van et al. 2013). Chitosan nanoparticles effected strongly on nutrient uptake of the coffee seedlings in the green house condition. In the same cultivation condition, application of chitosan nanoparticles enhanced significantly the uptake of nitrogen, phosphorus, potassium, calcium and magnesium compared to the control (Van et al. 2013). In Robusta coffee nanochitosan substantially expanded chlorophyll content material, photosynthetic depth, nutrient uptake, and seedling growth (Van et al. 2013). Zn–chitosan formulation has been evaluated for growth and development in dry bean (Ibrahima and Ramadan 2015). The effect of foliar application of nanochitosan NPK fertilizer at the chemical composition of wheat grains. Foliar application of nanofertilizers showed a sizable increase in total saccharide, while induced significant decrease in protein content and nitrogen content of the wheat grains (Abdel-Aziz et al. 2018).

The growth promoting impact of micro-chitosan has been verified to be significantly lower as compared to chitosan NPs. The growth promotory effect of bulk chitosan has been recorded significantly lower as compared to chitosan NPs. Similarly, as compared to control and CuSO<sub>4</sub>, bulk chitosan has been reported to have higher value for all parameters except for percent germination (Saharan et al. 2016). Similar efforts are needed for nanomaterials intended for use as fertilizers, so that nanofertilizers are advanced from the current mostly pristine products easily manipulated by the test environment to more functional products. To this quit, upgrades to this point made to nanoscale nutrients to generate improved nanofertilizers consist of the ones already stated in following sections concerning surface modifications along with alginate and chitosan (Saharan et al. 2016; Abdel-Aziz et al. 2016). There is an pressing neediness to make the most chitosan-based nanoparticles alone or in conjugation with various organic and inorganic compounds. Chitosan biopolymer based totally nanoparticles have large plant growth stimulatory activity (Table 13.2).

Very few studies have implemented chitosan particles in the agricultural context including incorporation of NPK fertilizer into the chitosan nanoparticles to make fertilizer consumption more efficient (Corradini et al. 2010; Wu et al. 2008; Hasaneen et al. 2014). Nanoparticles composed with an internal coating of CHT, an outer coating of cross-linked poly(acrylic acid)/diatomite-containing urea, and a middle of water-soluble granular nitrogen (N), phosphorus (P), and potassium (K) (NPK) fertilizer showed controlled release of the nutrients with no adverse impact

on the soil (Wu et al. 2008). Recently, biodegradable polymeric chitosan NPs (~78 nm) were used for controlled release of the NPK fertilizer sources, which includes urea, calcium phosphate, and potassium chloride (Corradini et al. 2010), while Hussain et al. (2012) encapsulated urea in CHT microspheres acquiring a controlled release of the nutrient.

Chitosan NPs of the mean diameter 20 nm for loading NPK fertilizers were prepared and showed that the stability of the colloidal chitosan nanoemulsion was better with the addition of nitrogen and potassium than with the addition of phosphorus because of the higher anion rate from the calcium phosphate than the anion charges from the potassium chloride and urea (Hasaneen et al. 2014). Biodegradable and biocompatible nanoparticles in a green manner originated from locally available raw materials and natural excipients addressing the said risks which will ultimately lead to development of eco-friendly nanofertilizers to release nutrients gradually in a controlled manner was produced. A herbal pass linker, Genipin was extracted from tender fruit of *Gardenia jasminoides* (Shantha Siri et al. 2017).

### 13.4.9 Pesticides Delivery and Remediation

Nano-pesticide transport systems have a series of critical advantages over their classical bulk counterparts. Chitosan has emerged as one of the most promising polymers for the green transport of agrochemicals along with micronutrients, insecticides, and herbicides in nanoparticles. Chitosan simply adsorbs to plant surfaces (e.g., leaves and stems), which helps to prolong the touch time between agrochemicals and the goal absorptive surface. Chitosan nanoparticles are seen to facilitate active molecule or compound uptake through the cellular membrane. The absorption improving impact of chitosan nanoparticles improves the molecular bioavailability of the active elements contained inside the nanoparticles (Tiyaboonchai 2003).

Polyvinylpyridine and polyvinylpyridine-co-styrene nanoparticles were investigated to control release of tebuconazole and chlorothalonil fungicides for better wood maintenance (Liu et al. 2001). Boehm et al. (2003) developed strong polymeric nanospheres (135 nm) with 3.5% encapsulation rate, and apart from the low active ingredient content, this formulation yielded substantial upgrades in the bioavailability of the insecticide (RPA 107382) to crops. It has additionally been reported that aluminosilicate-crammed nanotubes stick to plant surfaces whilst the nanoscale aluminosilicate particles leach from the nanotubes and in the end stick to the surface hair of insect pests. These particles ultimately enter the body and influence certain physiological functions (Bhattacharyya et al. 2010; Kashyap et al. 2013). A new type from amphiphilic derivative of chitosan, N-(octadecanol-1-glycidyl ether)-O-sulfate chitosan was produced by Lao et al. (2010). Nanoparticulate structures based totally on herbal polysaccharides (chitosan and cyclodextrin) have been prepared for use in the ionic gelation technique and have been used by companies for botanical pesticides, including carvacrol and linalool (Campos et al. 2018).

Some authors synthesized and tested changed CHT nanoparticles to hold paraquat, the most widely used herbicide. Cea et al. (2010) included atrazine, a herbicide used for broadleaf weed control, into ethyl cellulose managed release formulations (CRFs) with the aid of solvent evaporation. Allophanic clays and nano-clay changed the matrix. All CRFs multiplied the atrazine activity and decreased leaching loss. Silva et al. (2011) verified that alginate/CHT nanoparticles regulate the discharge of the herbicide and its interaction with the soil. In addition, CHT/tripolyphosphate nanoparticles decreased paraquat toxicity (Grillo et al. 2014), improved herbicidal activity against *Eichhornia crassipes* was observed when paraquat was encapsulated in silver/CHT nanoparticles (Namasivayam et al. 2014). Decomposable chitosan-lactide copolymer was implemented as a hydrophobic provider for pyraclostrobin, a wide-spectrum foliar fungicide. In comparison with 25% pyraclostrobin emulsifiable lister, the NPs confirmed higher fungicidal interest against *Colletotrichum gossypii* (Xu et al. 2014). More recently, nanoparticles of CHT and sodium tripolyphosphate have been prepared and evaluated for suppression of *Pyricularia grisea* (Manikandan and Sathiyabama 2016) and oleoyl-chitosan nanoparticles have been synthesized and used to disperse antifungal products (Xing et al. 2016).

The authors concluded that these novel absorbent materials may be implemented as an alternative biocompatible and green method for pesticide removal, and also applied in water remedy systems. Bin Hussein et al. (2009) had already evolved a unique nanohybrid pesticide controlled release machine from 4-(2, four-dichlorophenoxy) butyrate and a Zn–Al-layered double hydroxide inorganic inter-layer with the aid of specific methods. The bioavailability of the chiral herbicide dichlorprop to the green alga *Chlorella pyrenoidosa*, in the absence and presence of chitosan nanoparticles was reported by Wen et al. (2010). The capacity of debris primarily based on polymers and cyclodextrin to adsorb insecticides is indicative of their capability for use in water remediation (Liu et al. 2011). Chitosan complexes and nanoparticles of chitosan containing the chiral herbicide dichlorprop were synthesized and characterized with the aim of decreasing potential for leaching and contamination of subterranean waters (Wen et al. 2011). Celis et al. (2012) used specific methods to prepare a bionanocomposite cloth based totally on chitosan and clay (montmorillonite), used as an adsorbent for the herbicide clopyralid found in an aqueous solution or in a mixture of water and soil. A nanocomposite material of CHT and montmorillonite was used to adsorb and dispose of the herbicide clopyralid existing in water and soil (Grillo et al. 2014). Chitosan–zinc oxide (CS–ZnO) nanoparticles as an absorbent for the elimination of the pesticide permethrin from water. ZnO nanoparticles possessed an nearly spherical morphology with a size of 58 nm. Researchers investigated the influence of the quantity of absorbent, agitation time, pesticide initial concentration, and pH on the sorption of the pesticide by using CS–ZnO absorbents (Dehaghi et al. 2014). In addition, CHT/tripolyphosphate nanoparticles decreased paraquat toxicity (Grillo et al. 2014), (Namasivayam et al. 2014). Alginate/chitosan and chitosan/tripolyphosphate nanoparticles were developed for encapsulation of the herbicides imazapic and imazapyr. Comparison was made between the nanoparticle systems and the free herbicides in phases of their

**Table 13.3** Applications of nanochitosan for removal of pesticides

Carrier/polysaccharide	Active principle	Class	Reference
Alginate-chitosan nanoparticles	Paraquat	Herbicide	Silva et al. (2011)
chitosan/tripolyphosphate	imazapic and imazapyr	Herbicide	
Chitosan nanoparticles and chitosan	Dichlorprop	Herbicide	Wen et al. (2011)
Chitosan	Dichlorprop	Herbicide	Wen et al. (2010)
Montmorillonite-chitosan bionanocomposites	Clopyralid	Herbicide	Celis et al. (2012)
Copper chitosan nanocomposites	organophosphorous	Pesticide	Jaiswal et al. (2012)
Chitosan nanoparticles	Hexavalent chromium	Metal	Geng et al. (2009)
Chitosan/ $\beta$ -cyclodextrins Films	Carvacrol	Insecticide	Higueras et al. (2013)
Chitosan	–	–	Alves and Mano (2008)
Chitosan	NPK	Fertilizer	Wu and Liu (2008)
Chitosan	–	–	El-Sawy et al. (2010)
Chitosan	Rotenone	Insecticide	Lao et al. (2010)
Chitosan	1-Naphthylacetic acid	Hormone	Tao et al. (2012)
Chitosan-silver nanoparticles	–	pesticide	Saifuddin et al. (2011)
Chitosan NPs/ $\beta$ -cyclodextrins	Carvacrol and linalool	pesticide	Campos et al. (2018)

cytotoxicity and genotoxicity that allows to verify that the encapsulation procedure led to a reduction in toxicity (Maruyama et al. 2016). These observations delivered a clear suggestion that chitosan was able to modify the enantioselective bioavailability of the pesticide and herbicide, which could be safe for agricultural ecology and environment (Table 13.3).

### 13.4.10 Plant Genetic Transformation

Nanotechnology will possibly play a critical role in the improvement of genetically modified plants. The improvement of biotic and abiotic kinds of crop plant involves the transport of genetic cloth of both DNA and RNA resulting in the alteration of gene expression (Palerice and Gatehouse 2008). There are many obstacles for gene transfer to transform plant (Ghormade et al. 2011). Gene transfer is a technique for plant transformation using nanoparticle through nonviral-mediated transport vehicles including chitosan nanoparticle. And it's better than traditional methods in plants such as Agrobacterium-mediated gene transfer, electroporation, PEG-mediated gene transfer, particle gun bombardment, etc., are costly, labor intensive and cause significant perturbation to the growth of cells. In addition, these methods have very low efficiency (0.01–20% performance). However, it has been particularly a success for genetic transformation of dicots (Sivamani et al. 2009).

Biodegradable nanoparticles with common diameters of approximately 0.2  $\mu\text{m}$  can be a product of chitosan and polyglutamic acid and used as microprojectiles (Lee et al. 2008). These particles had been effective for encapsulating and protecting DNA for transdermal gene transport by way of acceleration with a low-strain gene gun. Yu-qin et al. (2012) have screened the benefits of using nanoparticles over conventional companies. Firstly, nanoparticles are applicable to both monocotyledons and dicotyledonous plants and any types of organs. Secondly, this type of gene carriers can effectively overcome transgenic silencing via controlling the copies of DNA combined to nanoparticles. Thirdly, nanoparticles can be easily functionalized so as to further enhance transformation efficiency. Finally, nanoparticles-mediated multigene transformation can be achieved without involving traditional building method of complex carrier.

Biodegradable polymers like chitosan can report an advantageous charge on DNA conjugate nanomaterial surface (Li et al. 2011). Chitosan is a polymer that has been used extensively both in nucleic acid delivery and tissue engineering packages (Rafferty et al. 2013). CHT nanoparticles can appropriately deliver genetic material for plant transformation (Malerba and Cerana 2016). Chitosan/DNA nanoparticles can be effectively formed by using coacervation among the definitely charged amine corporations on chitosan and negatively charged phosphate groups on DNA. However, the transfection performance of chitosan is low. The transfection performance has been shown to rely on the chitosan molecular weight, degree of deacetylation, pH of the transfecting medium, and cell kind (Mao et al. 2010). A pH of 6.8–7.0 is vital for transfection (Sailaja et al. 2013), and proof shows that DNA complexes shaped through shorter and near monodisperse chitosan oligomers (24-mer) have extra applicable properties than ultrapure chitosan and are therefore extra attractive as gene shipping systems than the traditional high molecular weight chitosans (Koping-Hoggard et al. 2003). Besides this, the degree of chitosan deacetylation additionally acts as an important aspect in chitosan–DNA nanoparticle method because it impacts DNA binding, launch, and gene transfer performance in vitro and in vivo assays (Kiang et al. 2004).

In particular, the ability of use of CHT NPs for genetic transformation is proposed by using its capability to shape, via electrostatic interactions, a complicated where DNA is included from nuclease degradation (Mao et al. 2010; PichyaIriti and Varoni 2015). Interesting effects were obtained by Wang et al. (2013), who devolped QD-labeled CHT-DNA complexes to display nanoparticle-mediated genetic transformation of cultured cells of *Jatropha curcas*. This approach gave an upward push to solid transformants with higher performance than other conventional strategies of gene transfer. Recent data confirmed that primarily CHT-based nanoparticles with highly positive surface coatings can passively penetrate throughout the chloroplast membrane.

Once in the chloroplast, these nanoparticles showcase both restricted diffusion and convection before attaining an irreversibly trapped state (Wong et al. 2016). These results propose that CHT nanoparticles may be used as viable molecular transporters into plastids, just like the chloroplast. Fungal resistance of transgenic potato flowers expressing thionin genes isolated from Brassicaceae species

(*Arabidopsis thaliana*) was evaluated against the phytopathogenic fungi. Thionins inhibit the increase in vitro of approximately 20 one-of-a-kind fungal plant pathogens that include *Botrytis cinerea*, *Fusarium* spp., *Phytophthora infestans*, and *Rhizoctonia solani* (Cammue et al. 1992). It is obvious that protein extract of five replicates of transgenic potato that incorporate thionin proteins showed antifungal activity against some pathogenic fungi with reduction in mycelial growth, illustrate the inhibitory effect of protein extract of the transgenic potato cultivars on radial growth of the PDA plates of four different pathogenic fungi after incubation for 7 days at 28 °C compared with control treatment (Abdel-Razik et al. 2017).

Two thionin resistance genes have been transferred into potato cultivars using chitosan nanoparticle, and then examined the resistance of transgenic species with the fungal pathogens *Alternaria alternata* and *Rhizoctonia solani* (Abdel-Razik et al. 2017). Recently, chitosan has attracted sizeable attention for use in formulations with small interfering RNA (siRNA). Because of the cationic nature, chitosan could make complex with siRNA in nanoparticles profile. Some studies suggest the utility of chitosan nanoparticle-entrapped siRNA as a service for siRNA delivery (Ragelle et al. 2013). Zhang et al. (2010) have proven that chitosan nanoparticles successfully added dsRNA (against chitin synthase genes) in stabilized shape to mosquito larvae through feeding. Chitosan nanoparticles must verify to be effective in dsRNA delivery due to their efficient binding with RNA, protection, and the ability to penetrate through the cellular membrane. These effects really suggest that chitosan nanoparticles primarily based siRNA formulations may additionally make contributions to plant pathogen and pest control while warding off the lengthy method of conventional plant transformations. There are a few mixed consequences with reference to gene transport through chitosan nanoparticles, but given the potential benefits of chitosan nanoparticles to assist within the transfer of genetic material to design new and enhanced plant genotypes (Kashyap et al. 2015).

### 13.5 Future Prospects

Therefore the incorporation of natural antimicrobial compounds in based-chitosan release matrices as micro- and nanoparticles is a topic of interest for future research. This may represent a promising alternative for use in the control of bacteria, fungi and insects in pre- and put up-harvest foods, contributing to the eradication of the troubles related to the usage of chemicals on food crops (Cota-Arriola et al. 2013). However, nanotechnology can both enhance crop productivity and reduce nutrient losses. As demonstrated in the course of this chapter, the nano-chitosan have been widely used in research in order to develop new formulations with active compounds of interest in plant protection. These formulations not only release the active compound in a slow manner but also after degradation increase the crop output besides proving the water-holding capacity of the soil. Cu and Zn have traditionally been used in agrochemicals. Cu and Zn, blended with chitosan may additionally reduce the threat of hazardous agrochemicals for crop improvement and protection.

Chitosan nanofertilizer assessments should be done using mixtures of nanoscale nutrients to mimic conventional fertilizer application regimens typically involving multiple nutrients applied simultaneously (e.g., NPK).

## 13.6 Conclusions

The role of chitosan NPs in the agricultural sector is highlighted as insecticides and growth promoters for plants, and additionally as preservatives in the course of post-harvest and packaging of agriproducts. Studies of those nanomaterials are specifically focused on components of in vitro control, so it is important to perform in situ control assessments to offer answers and alternatives to the problems that the agricultural field faces. Additionally, the numerous makes use of nanochitosan inside the plant protection turned into mentioned, beginning with the utility of it as a pesticide remediation and gene delivery for the plants and controlling post-harvest diseases. The shape of chitosan NPs and their impact is determined by their type, concentration, mixture (monomer or combined with other compounds), and response (temperature and time). However, this biopolymer requires investigation and further exploration to better understand its multifunctionality, properties, and mechanisms. Use of nanochitosan for transport of agrochemicals (insecticides, micronutrients, fertilizers, and plant growth hormones) would be the most promising discipline in the coming years for nanotechnology utility in agriculture. In conclusion, chitosan formulations may be used for more secure use of nano-agrochemicals.

**Acknowledgment** This research was supported by the Science and Technology Development Fund (STDF), Joint Egypt (STDF)–South Africa (NRF) Scientific Cooperation, Grant ID. 27837 to Kamel Abd-Elsalam.

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