



REVIEW ARTICLE

Chitosan-based nanoformulations for sustainable plant disease management: From biopolymer to nano-enabled protection

Bhavya Prasad Nair¹, Johnson Iruthayasamy^{1*}, Karthikeyan Muthusamy¹, Arunprakash Soodamani¹,
Raja Kalimuthu² & Chandrakumar Kalichamy³

¹Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

²Department of Seed Science and Technology, Agricultural College and Research Institute, Chettinad 622 209, Tamil Nadu, India

³Department of Biochemistry, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Correspondence email - johnson.i@tnau.ac.in

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Abstract

Plant pathogens pose persistent threats to global food production, driving the need for sustainable and effective disease management strategies. While the use of chemical pesticides is the primary control measure adopted, their environmental toxicity has raised concerns, prompting extensive research into the development of eco-friendly alternatives. Nanotechnology has emerged as a promising and sustainable tool for plant protection. The biopolymer chitosan is well-known for its unique physicochemical properties including biodegradability, biocompatibility and low toxicity, making it one of the most reliable polymers for nanoformulation development. These formulations, including nanoparticles and nanocomposites, incorporate chitosan as an antimicrobial nanomaterial as well as a carrier for other biocidal nano-substances offering an efficient solution against plant diseases. Chitosan as a carrier enhances the targeted delivery of the encapsulated substances through their slow and controlled release. The nanoformulations adopt multiple modes of action to combat pathogens, which include membrane disruption, inhibition of growth and biofilm formation and induction of defence responses within the plant. This review elucidates the advantages of using chitosan for fabricating novel nanoformulations for plant protection. Their superior modes of action against fungi, bacteria, viruses and nematodes have also been highlighted alongside challenges such as stability and scalability, thus paving the way for their integration into sustainable pest management systems.

Keywords: chitosan; nanocomposite; nanoencapsulation; nanoformulation; plant defence; plant protection

Introduction

Global population levels continue to expand at a rapid rate. This rapidly surging demographic pressure demands higher food production to address concerns regarding food security. However, limited arable land resources have necessitated intensive cropping practices, leading to increased incidence and severity of pest and disease outbreaks, resulting in severe yield losses (1). These challenges are further aggravated by climate change that alters the life cycle of pathogens, their vectors and host-interactions. Collectively, these challenges highlight the urgent need for resilient and sustainable plant protection strategies.

Being a critical constraint to crop production, pest and disease epidemics have compelled producers to heavily rely on the use of synthetic chemicals due to their rapid and broad-spectrum activity. However, the indiscriminate use of these synthetic chemicals has raised several concerns with respect to environmental and human health. These chemicals adversely affect a wide range of non-target organisms such as pollinators, earthworms, natural enemies, rhizosphere colonising organisms and other biological control agents (2). Moreover, toxic residual accumulation over the

years causes an overall deterioration of soil quality. When such residues enter the food chain, they pose serious risks to humans and cattle. Direct exposure to these chemicals during application is also highly hazardous, resulting in health complications such as cancer and several respiratory, reproductive and neurological disorders (3). Beyond environmental risks, the continuous application of high doses of agrochemicals can induce resistance in pathogenic organisms, leading to the evolution of highly virulent strains. Such mutations may result in a higher incidence of the disease rather than its suppression (4). These factors demand the development and adoption of other eco-friendly strategies.

Nanotechnology has emerged as a promising and innovative platform for improving plant health and managing disease severity. Nanomaterials possess dimensions typically ranging 1–100 nm (5), with a relatively high surface area to volume ratio, resulting in improved reactivity and aiding easier absorption into the biological systems. These attributes confer nanoparticles to effectively suppress pathogens through various mechanisms including disruption of microbial membranes, oxidative stress induction, inhibition of biofilm formation and interference with metabolism (6). However, the direct application of nanoparticles is

countered by challenges related to their stability, aggregation, evaporative losses and phytotoxicity at high concentrations (5, 7). To overcome these limitations, biopolymer-based nanoformulations particularly those utilising chitosan as a carrier have gained considerable interest. Chitosan is a biodegradable and non-toxic derivative of chitin and it possesses inherent antimicrobial and plant growth-promoting properties. Chitin can be easily derived from marine waste, but it is insoluble in water, organic solvents, dilute acids and alkali (7). In contrast, chitosan is a cationic polymer which can dissolve in organic solvents like mild acids. The cationic nature and the presence of highly reactive amino and hydroxyl functional groups enable the encapsulation of bioactive substances within the chitosan shell, thus offering a synergistic control over the pathogens (8, 9). Chitosan-based nanoformulations have thus been proven to improve stability, controlled release and targeted delivery of the active components and provide a broad-spectrum suppression of pests and diseases. Although numerous studies have demonstrated their antimicrobial and defence eliciting properties, most of them involve only laboratory scale evaluation and limited field level studies. Critical aspects such as long-term environmental fate, interaction with beneficial microbes and scalability also remain less explored. Therefore, a comprehensive application-oriented evaluation of chitosan-based nanoformulations is essential to bridge the gap between existing studies and practical use.

Chitosan

Origin and chemical structure

Chitosan is a naturally occurring biopolymer, derived from the partial deacetylation of chitin. Chitin is the second most abundant biopolymer on earth after cellulose. It is widely distributed in nature and is a primary constituent of the exoskeletons of arthropods and marine crustaceans, as well as the cell walls of algae and fungi (10). Charles Rouget derived chitosan from chitin by its alkaline deacetylation, leading to its identification as a distinct biopolymer in the year 1859 (11). The strong inter-molecular and intra-molecular hydrogen-bonding between the N-acetylglucosamine (NAG) units of chitin makes it insoluble in common solvents like water, leading to the development of chitosan as a more soluble alternative (12). Chitosan is composed of alternating units of N-acetylglucosamine and glucosamine linked by β -(1 $\rightarrow$ 4) glycosidic linkages and is

formed by partial deacetylation of chitin (7). This biopolymer can be engineered into desired forms and utilised for diverse research and agricultural applications (Fig. 1).

Although chitosan can be produced from different chitin-containing raw materials, its total productivity varies considerably depending upon the source and extraction procedure. The most common sources include crustacean products such as shrimp shells yielding approximately 15.4 % chitosan followed by 13.3 % from crabs and 12.6 % from squilla (13). In addition, chitosan recovery percent of upto about 96 % has been reported in insects (14). Fungal biomass also represents a promising source reporting chitosan yield ranging 2.7–15.7 % (15).

Physicochemical properties of chitosan relevant to nanoformulations

Solubility

Chitin is insoluble in solvents like water due to the strong intermolecular and intramolecular hydrogen bonding between its N-acetylglucosamine units (7). During its deacetylation process that produces chitosan, some of its N-acetylglucosamine units get converted into glucosamine units. Such a partial deacetylation of about 50 % enhances the solubility of the compound in mild acids (16). Chitosan requires a lower pH of 4–5 to exhibit maximum dissolution, as an acidic environment leads to protonation of its amino groups (17). Deprotonation occurs at higher pH values, reducing solubility and promoting precipitation. The acid dissociation constant (pKa) of chitosan falls around 6.5, which means the maximum solubility can be achieved at a pH less than 6.5. Despite formic acid being the best solvent for dissolving chitosan, mild concentrations of acetic acid are the most used standard solvent for the same. While acids such as hydrochloric acid and nitric acid can also dissolve the polymer, certain others like phosphoric acid and sulphuric acid result in its precipitation and hence insolubility (18). The higher the degree of deacetylation, the higher the solubility.

Molecular weight

Chitosan can be classified into three different types, namely, low molecular weight (< 100 kDa), medium molecular weight (100–1000 kDa) and high molecular weight (> 1000 kDa) chitosan. Among these, low molecular weight chitosan has wider biological

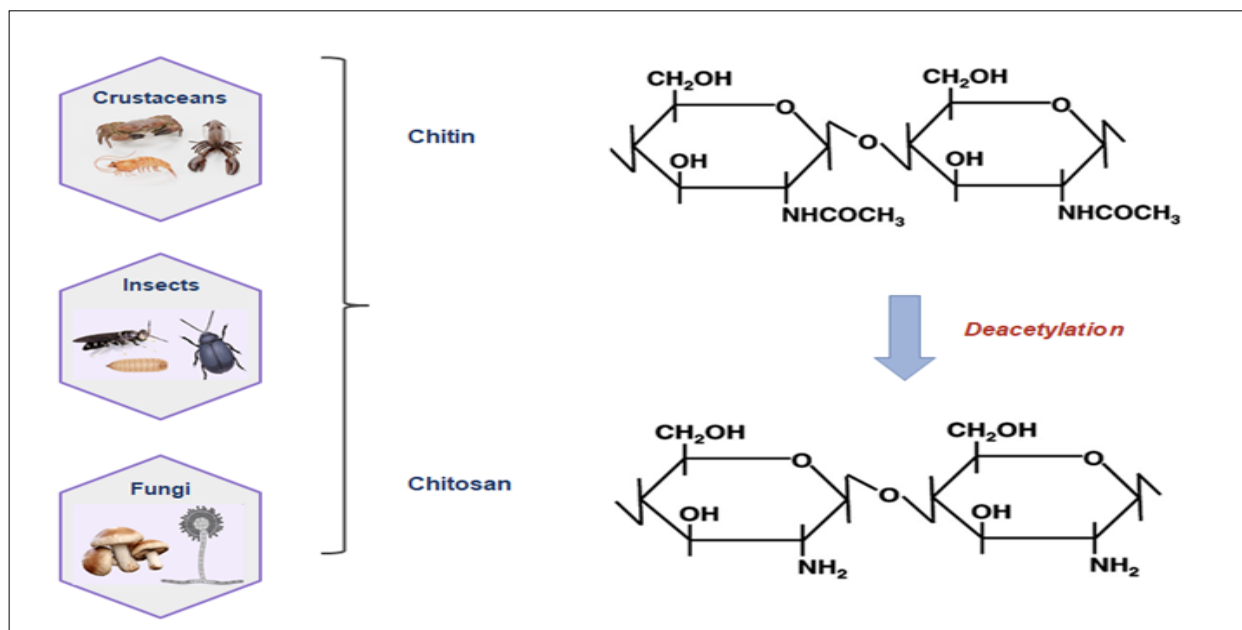


Fig. 1. Sources of chitin and its conversion to chitosan.

applications, due to its superior solubility in standard solvents relative to high and medium molecular weight variations (19). This improved solubility is associated with greater availability of protonated groups and in turn a much higher degree of deacetylation which enhances polymer-solvent interactions (20). Chitosan with a molecular weight in the range of 22–30 kDa was found to exhibit moderate solubility even in water (17). Moreover, low molecular weight chitosan has been frequently found to exhibit superior antimicrobial activities and plant growth promotion. A significant enhancement in vegetative growth and fruit yield in chilli has been reported with the use of low molecular weight chitosan. It was concluded that the low molecular weight chitosan performed better due to its ability to cross external barriers and interact with cellular components more easily (21).

Viscosity

The viscosity of chitosan solution is directly proportional to changes in its molecular weight and the degree of deacetylation. This measure can be employed as an indirect indicator of the stability of chitosan-based formulations in storage conditions, as their viscosity gradually decreases upon storage. As chitosan is a biodegradable polymer, it is susceptible to hydrolytic cleavage, leading to a reduction in molecular weight and viscosity. Such a reduction in the viscosity might subsequently affect the stability of chitosan-based formulations (22).

Functional groups and reactivity

The chemical structure of chitosan is characterised by the presence of primary amino groups at the C-2 position and hydroxyl groups at the C-3 and C-6 positions. The amino groups serve as the sites of protonation under acidic conditions and enable strong electrostatic interactions with negatively charged biomolecules. Meanwhile, the hydroxyl groups contribute to intra and intermolecular hydrogen bonding which maintains the structural integrity of chitosan chains. The protonation of its amino group leads to repulsion between individual chains of chitosan and disruption of H-bonding, causing its dispersion in the acidic environment (23). The tendency to react with negatively charged molecules imparts a strong metal-chelating ability to chitosan through coordination interactions, leading to the formation of complexes with ions such as copper (Cu^{2+}), silver (Ag^+), nickel (Ni^{2+}), or zinc (Zn^{2+}), adding to its antimicrobial potential by depriving pathogens of their essential nutrients. In soils affected by heavy metal contamination, chitosan and its derivatives can be employed as promising candidates for metal immobilisation and detoxification (11, 24). Hence, the high reactivity of chitosan's functional groups highlights its role in nanoformulation development and plant protection applications. Collectively, the physicochemical attributes of chitosan mentioned above are crucial in determining its behaviour and compatibility during nanoformulation, influencing the key features such as particle size, zeta potential, stability and interaction with foreign molecules.

Chitosan-based nanoformulations

The versatile physicochemical properties of chitosan make it a promising biopolymer for plant protection applications. Though effective in crop protection, the bulk form of chitosan is accompanied by extreme difficulty in converting it into a practical formulation. However, the nature of the compound allows for its modification into more stable and functionally efficient nanostructures by nano-engineering. Consequently, the concept of chitosan-based nanoformulations has garnered considerable

attention in the field of plant pathology.

According to the International Organization for Standardization (ISO) and the International Union of Pure and Applied Chemistry (IUPAC), nanoparticles are defined as materials that fall within the diameter range of 1–100 nm (5). However, in agricultural nanotechnology research, the size range of nanoparticles and nanoformulations is usually extended upto 1000 nm. This flexibility in the size criterion is common for polymeric nanoformulations like chitosan. Such formulations are often functionally accepted as nanoformulations, owing to their ability to exhibit nano-specific properties including high surface area-to-volume ratio, enhanced interaction with biological membranes and controlled release behaviour, which distinguish them from their bulk counterparts (25). Apart from particle size determination, the comprehensive characterisation of formulations using Fourier-transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy and zeta potential analysis remains important for establishing them as nanoformulations.

Chitosan nanoparticles

Chitosan nanoparticles represent the simplest and most extensively studied nano-form of chitosan for plant protection. The difficulty in solubilising chitosan hinders its use in direct sprays. The integration of chitosan's inherent antimicrobial and elicitor properties with nanotechnology significantly enhances its efficiency through improved solubility, reactivity and interaction with microbes. Although chitosan nanoparticles were originally developed for chemotherapeutic drug delivery, their application in plant protection has gained momentum due to their broad-spectrum activity against pathogens. Chitosan nanoparticles can be prepared using various top-down or bottom-up approaches, mainly ionotropic gelation, reverse micellar method, droplet coalescence, emulsion crosslinking and spray drying. Among these, ionotropic gelation has emerged as the most widely adopted method for nanoparticle synthesis due to its simplicity and better control over particle size. In this method, electrostatic interactions between protonated amino groups of chitosan and polyanions such as sodium tripolyphosphate generate stable nanoparticles, typically within the range of 10–200 nm. Particle size can be effectively modulated by adjusting the concentration of chitosan and crosslinkers like sodium tripolyphosphate, which directly influence plant uptake and bioactivity (26).

Smaller particles exhibit enhanced penetration and systemic transport within plant tissue. Chitosan nanoparticles with an average size of 100–156 nm demonstrated significant suppression of *Harpophora maydis* in maize while improving its yield parameters compared to bulk chitosan treatments (27). Similarly, broad spectrum antifungal activity of chitosan nanoparticles has been reported against a diverse range of ascomycetes and oomycetes even at a very low concentration of 0.05 % (28). The enhanced antifungal performance of nanochitosan has also been linked to direct ultrastructural damage to fungal cells, including pronounced hyphal deformation, membrane disruption and cytoplasmic leakage. These effects are widely attributed to strong electrostatic interactions with the negatively charged phospholipid membranes, ultimately compromising cellular homeostasis (29). Chitosan also functions as a potent elicitor of plant defence responses. The application of chitosan nanoparticles induces significant upregulation of pathogenesis related (PR) genes in plants (30). This nanoparticle-mediated activation of defence signalling enhances

the production of antimicrobial enzymes, thereby promoting pathogen inhibition. Apart from the plant protection aspects, nanochitosan serves as an important candidate for promoting root and shoot development, enhancing photosynthesis, improved nutrient absorption and metabolism and stimulating the production of metabolites such as alkaloids and phenolics (31). Despite these advantages, the efficacy of chitosan nanoparticles can considerably vary depending on factors such as particle size, molecular weight, degree of deacetylation and surface charge, highlighting the need for standardised synthesis and characterisation.

Chitosan-metal nanocomposites

Chitosan-based metal nanohybrids have gained momentum in combating plant pathogens. A wide range of metal particles possess intrinsic antimicrobial properties. They act against persistent pathogens in a multifaceted manner including enzymatic inhibition, membrane disruption, interference with nucleic acid metabolism and oxidative stress induction. Such metal and metal oxide-based chitosan nanocomposites can be fabricated by chemical methods or by green synthesis using plant and fungal extracts (26).

Silver has long been recognised for its cleansing and antimicrobial properties. Studies report that silver nanoparticles effectively suppress a wide range of phytopathogens. The potential of these metal particles in plant protection can be maximised by integrating them as chitosan-based nanoformulations. Chitosan-silver (CS-Ag) nanocomposites synthesised using the endophytic fungus *Curvularia kusanoi*, isolated from *Juniperus procera* Hochst. ex Endl. controlled the growth of *Curvularia lunata*, *Cladosporium* sp. and *Aspergillus fumigatus* with the highest inhibition observed against *C. lunata* (32). Similarly, the same formulation suppressed appressorium formation, melanin production and sporulation in *Colletotrichum truncatum* (33). The pronounced efficacy of these formulations is believed to be linked to the combined action of Ag⁺-induced enzyme disruption and chitosan-driven membrane destabilisation, chelation of essential ions and transcriptional suppression.

Similarly, copper and copper oxide nanoparticles have also found their application in plant disease management. Moreover, copper forms an important constituent of various fungicides and bactericides (34). Thus, chitosan-copper (CS-Cu) nanoformulations represent another class of widely investigated nanocomposites in pathogen control and are less toxic compared to synthetic copper-based fungicides. These systems have been shown to impair hyphal growth and drastically reduce melanisation in certain fungi, while hindering sclerotial germination in organisms like *Rhizoctonia solani* (35).

Apart from silver and copper, chitosan-based nanocomposites incorporating other essential metals such as zinc, titanium and iron and their oxides have also been studied. Chitosan-metal oxide nanocomposites such as chitosan-zinc oxide (CS-ZnO) nanocomposites reduced *Fusarium oxysporum* f. sp. *ciceri* inoculum in chickpea with enhanced root nodulation (36). In another study, chitosan-zinc formulation surpassed carbendazim in controlling *Macrophomina phaseolina*, while boosting defence enzyme activity (37). A notable increase in total protein content, especially ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) indicating improved photosynthetic performance was recorded on treatment of rice blast affected plants with nickel-chitosan nanocomposites (38). The protective effects of other similar nanocomposites have been

discussed in Table 1. However, concerns regarding the fate of metal nanoparticles, their accumulation in the soil and possible effects on non-target organisms require further investigation before large-scale application. Collectively, these findings confirm that metallic chitosan nanocomposites function as versatile plant protective agents by simultaneously targeting pathogens and strengthening host defence responses.

Essential oil-chitosan based nanocomposites

Essential oils are among the widely explored plant-derived antimicrobials extracted from various plant parts including seeds, bark, leaves, roots and fruit peels. This explicit biological activity is attributed to the diverse combination of terpenoids and phenylpropanoids such as thymol, carvacrol, eugenol, citral, menthol, linalool and so on, present in them (42). Despite their effectiveness against phytopathogens, the practical use of these essential oils is constrained by rapid volatilisation and sensitivity to harsh environmental factors. Thus, their integration as nanoformulations is important to enhance their targeted delivery. These oil-based nanoformulations are commonly developed as sprayable nanoemulsions or fruit-coating films.

Encapsulation of these essential oils within chitosan matrices reduces the unintended loss of the active substances. For instance, chitosan-encapsulated essential oils from *Carum carvi* L., *Carum copticum* (L.) C.B. Clarke, *Mentha aquatica* L., *Mentha piperita* L. and *Satureja khuzistanica* Jamzad. exhibited strong inhibition against charcoal rot of bean. Among these, nanoemulsions derived from *C. copticum*, *S. khuzistanica* and *C. carvi* extracts offered complete growth inhibition (43). Similarly, *Cymbopogon martinii* (Roxb.) W. Watson. essential oil-based chitosan nanoemulsions demonstrated superior suppression of *Fusarium graminearum*, a post-harvest pathogen, compared to the free essential oil. The nanoformulation minimised degradation of the bioactive constituents under diverse conditions and induced structural alterations in fungal cells. These alterations included membrane damage, vesicle formation, lipid peroxidation and reduction in the ergosterol level, ultimately leading to growth inhibition (44). Likewise, clove oil-chitosan nanoemulsions also showed pronounced antimycotic activity against the soil-borne fungi *R. solani* and *Sclerotium rolfsii*, when compared with free oil and non-chitosan nanoemulsions. They also interfered with the production of key lytic enzymes produced by fungi such as cellulases and pectinases which play an important role in colonisation of host tissues (45).

In addition to improving antifungal activity, chitosan encapsulation has been shown to enhance the physicochemical stability of essential oils. Garlic essential oil incorporated into chitosan nanoemulsions, retained its antimicrobial properties while exhibiting greater resistance to thermal stress without significantly affecting the crystallinity of the polymer matrix. These emulsions were effective against seed-borne fungi such as *Aspergillus niger*, *Aspergillus versicolor* and *F. oxysporum* suggesting their potential application as eco-friendly seed protectant fungicides (46). Although numerous studies have demonstrated the antimicrobial potential of these formulations, their actual performance must be evaluated under field conditions across diverse environmental settings. Furthermore, their antimicrobial efficiency may also be influenced by variations in plant genotype and oil extraction techniques. The role of these nanoformulations has been discussed in Table 2.

Table 1. Metal-chitosan nanocomposites

Type	Particle size (nm)	Highest percent inhibition (%)	Pathogens inhibited	Key findings	References
CS-Ag NC	33.82	97.00 89.00 83.00	<i>Curvularia lunata</i> , <i>Cladosporium</i> sp., <i>Aspergillus fumigatus</i>	Silver-chitosan nanocomposite synthesised from <i>Curvularia kusanoi</i> isolated from <i>Juniperus procera</i> twigs exhibited strong antifungal activity.	(32)
CS-Ag NC	4	100.00	<i>Colletotrichum truncatum</i>	Treatment resulted in spore inactivation and inhibition of appressorium and melanin formation.	(33)
CS-Cu NC	70–74	64.22 100.00 65.54	<i>Magnaporthe oryzae</i> , <i>Rhizoctonia solani</i> , <i>Phytophthora capsici</i>	Nanocomposite-treated fungal mycelium exhibited darker pigmentation compared to the control, indicating its deterioration.	(35)
CS-CuO, CS-ZnO, CS-Ag NC	350 441 348	46.67 40.00 33.33	<i>Fusarium oxysporum</i> f. sp. <i>ciceri</i>	Effective antifungal activity; among tested formulations, CS-CuO NPs showed the maximum inhibition.	(36)
CS-Ni NC	30–40	64.00	<i>Pyricularia oryzae</i>	Reduced occurrence of blast disease, followed by a rise in the total leaf protein content especially RuBisCo; accumulation of photosynthetic pigments.	(38)
CS-Fe NC	86	67.00	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Inhibited biofilm formation and motility; enhanced rhizospheric microbial population; activated defence-related genes (<i>OsPRs</i> , <i>OsSOD</i> and <i>OsAP</i>) elevated the photosynthetic pigment concentration.	(39)
CS-Mg NC	43	80.69 81.02	<i>Acidovorax oryzae</i> <i>Rhizoctonia solani</i>	Exhibited strong good bactericidal and fungicidal activity, causing cellular shrinkage and leakage of cellular components.	(40)
CS-Ag NC	11.33	91.00	Alfalfa mosaic virus	Phenol, proline and capsaicin content were increased in treated pepper plants, which also showed notable virus inhibition.	(41)

Ag: silver; CS: chitosan; Cu: copper; Fe: iron; Mg: magnesium; NC: nanocomposite; Ni: nickel; Zn: zinc.

Chitosan-based nanofilms for post-harvest preservation

Nanofilms are thin materials used for coating the surface of perishable agricultural produce, mainly fruits and vegetables with the aim of shielding them from spoilage caused by post-harvest pathogens. Typically, nanofilms comprise a biological polysaccharide, into which bioactive substances such as metal ions, enzymes, plant extract and many others can be incorporated in their nano-form (47). For the synthesis of such chitosan-based nanofilms, various techniques are selected based on the intended purpose and film characteristics. This includes solvent casting, electrospinning, sequential layer deposition and dip coating. For practical post-harvest applications, dip-coating and solvent casting methods are preferred as they allow uniform deposition of the nanofilm over irregular surfaces of fruits and vegetables. In the solvent casting method, chitosan along with the desired nanoparticles is dissolved in a suitable solvent in the presence of additives like glycerol to

improve its flexibility. Following this, the solution is dried on a uniform surface to form a thin film. For coating cereal grains such as rice and wheat, the electrospinning technique is employed (48). This uses a high voltage electric field to generate nanofibres that can be assembled onto a substrate to form a nanofilm. Another common method used is the layer-by-layer assembly, where two or more compatible materials are deposited in a sequential manner to form nanofilms of desired thickness (49, 50).

The performance of these nanofilms is largely influenced by the nature of the incorporated bioactives. Chitosan nanofilms enriched with spearmint (*Mentha spicata* L.) essential oil displayed notable suppression of post-harvest pathogens such as *Penicillium italicum* and *Penicillium digitatum* in citrus fruits, while preventing their oxidation caused by Ultraviolet (UV) exposure (51). In plum fruits, coating with ginger-chitosan nanofilm improved their shelf life by enhancing phenolic and antioxidant levels, thus reducing

Table 2. Chitosan-essential oil nanoemulsions

Essential oil source	Particle size (nm)	Highest percent inhibition (%)	Pathogens inhibited	Key findings	References
<i>Carum carvi</i> , <i>Carum copticum</i> , <i>Mentha aquatica</i> , <i>Mentha piperita</i> , <i>Satureja khuzistanica</i>	45–232.6	87.66 96.06 11.38 17.29 96.47	<i>Macrophomina phaseolina</i>	Compounds such as menthol, menthone, carvacrol and thymol disrupted fungal membranes and inducing oxidative stress.	(43)
<i>Cymbopogon martinii</i>	455–480	-	<i>Fusarium graminearum</i>	Membrane permeabilisation occurred due to lipid peroxidation induced by the nanoformulation, followed by a reduction in ergosterol content.	(44)
<i>Syzygium aromaticum</i>	49–86	79.20 50.60	<i>Rhizoctonia solani</i> , <i>Sclerotium rolfsii</i>	Inhibition of cellulolytic and pectinolytic activities by the fungi resulted in minimal plant infection. It also hindered RNA and protein synthesis.	(45)
<i>Allium sativum</i>	172–352	100.00	<i>Aspergillus niger</i> , <i>Aspergillus versicolor</i> , <i>Fusarium oxysporum</i>	The nanoformulations were thermally stable. Fungal inhibition progressed with cell membrane damage, mitochondrial dysfunction and inhibition of fungal efflux pump activity.	(46)

the risk of pathogen-induced fruit deterioration. It also preserved the natural colour and firmness of the fruit throughout the storage period (52). Similarly, guava leaf essential oil-based coatings rich in β -caryophyllene reduced infections in chilli caused by *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. It also elevated the activity of β -1,3 glucanase, phenylalanine ammonia lyase and polyphenol oxidase as defence mediators (53). Chitosan films loaded with chamomile and thyme extracts were effective in lowering *Penicillium expansum* infection in apples compared with the extracts applied independently (54). The addition of metal nanoparticles further strengthens the antimicrobial action. Silver-chitosan nanocoatings markedly reduced anthracnose incidence in mango, with much better performance compared to chitosan alone and even to the fungicide carbendazim (55). These films also help in maintaining the quality of agro-products by shielding them from UV radiation and premature oxidative damage. The thickness of the films can be customised based on the product to control their exposure to moisture and gases (51). However, the effectiveness of such nanofilms may vary depending on the commodity type and film composition. Excessive film thickness might adversely affect gaseous exchange and moisture regulation. In addition, prolonged exposure to high humidity may affect film integrity and performance. Collectively, nanofilms act as an effective barrier against early degradation of produce caused by pathogen attack, provided they are synthesised using standardised protocols (Table 3).

Chitosan as a carrier for biocontrol agents

Microbial biocontrol agents are beneficial microorganisms that suppress plant pathogens without relying on synthetic chemicals. They contribute to disease management through diverse mechanisms, namely antibiosis, hyperparasitism, competition for nutrients and enzymatic degradation of pathogens. They trigger salicylic acid and jasmonic acid mediated defence pathways, inducing systemic resistance in plants. Additionally, they promote plant vigour by producing growth-promoting metabolites (56). Nano-encapsulation of these microbes within chitosan matrices improves their stability, delivery and field persistence. As a biodegradable polymer with inherent antimicrobial properties,

chitosan enables controlled release of these organisms in the rhizosphere while shielding them from UV radiation, desiccation and temperature and pH extremes. This also enhances microbial adherence to roots and complements chitosan-induced defence priming. Because of its mild, non-toxic and cell-compatible conditions, ionic gelation using sodium tripolyphosphate is one of the most widely used techniques for biocontrol agent encapsulation within chitosan (57). Chitosan-coated electrospun bioplastic fibres have been shown to successfully incorporate *Bacillus subtilis*, with the cells retaining their viability even after prolonged storage for 90 days (58). Analogously, poly(L-lactic acid) (PLLA)-chitosan nanohybrids loaded with *Trichoderma asperellum* spores as a biocontrol dressing effectively suppressed the growth of *Phaeoemoniella chlamydospora* and *Phaeoacremonium aleophilum*, the causal agents of grapevine esca disease (59). Beyond cell encapsulation, chitosan nano-carriers are also suitable for the delivery of microbial metabolites. Nanoemulsions incorporating cell free extracts of *Streptomyces* sp. were successful in curbing infection caused by *R. solani* in tobacco. The nanoemulsion exhibited greater foliar adherence and suppression of microbial pathogenesis, compared to non-encapsulated suspension sprays (60). The successful encapsulation of microbes depends upon several factors, particularly the molecular weight, the moisture retention ability and the viscosity of chitosan. Despite these promising findings, environmental variations such as soil moisture, temperature, pH and native microbial population may influence microbial survival, release kinetics and colonisation efficiency. Furthermore, long-term storage requires proper optimisation to ensure the longevity of encapsulated organisms. Overall, chitosan-based nanoformulations enhance the functional reliability, stress tolerance and biocontrol efficacy of microbial bioinoculants, as summarised in Table 4.

Chitosan-based nanoformulations for double stranded RNA (dsRNA) delivery

Chitosan-based nanoformulations have emerged as promising carriers for RNA interference (RNAi)-based plant protection strategies. Since most viruses replicate through double-stranded RNA intermediates, RNAi has widely been employed against these organisms (62). The central dogma of molecular biology dictates the

Table 3. Chitosan-based nanofilms for post harvest preservation

Nanofilm type	Particle size (nm)	Zeta potential (mV)	Zeta potential (mV)	Pathogen inhibited	Key findings	References
Chitosan-spearmint essential oil nanofilm	252.3	21.6	21.6	<i>Penicillium italicum</i> , <i>Penicillium digitatum</i>	Oxygenated monoterpenes (carvone) enhanced the fungistatic activity; low film pH completely inhibited green mold incidence and significantly inhibited blue mold.	(51)
Chitosan-ginger essential oil nanofilm	265.8–370.03	–25 to –33	–25 to –33	<i>Monilia fructicola</i> , <i>Rhizopus stolonifer</i>	Membrane degradation and cellular leakage; chitosan-induced chitinase-mediated cell wall disruption.	(52)
Chitosan-guava leaf essential oil nanofilm	1687	58.8 ± 7.47	58.8 ± 7.47	<i>Colletotrichum gloeosporioides</i> , <i>Rhizopus stolonifer</i>	Weight loss, enzymatic browning and electrolyte leakage were reduced; fungal inhibition was observed while maintaining firmness, total soluble solids and phenolic content in fruits.	(53)
Chitosan-thyme extract nanofilm	20–50	20–50	-	<i>Penicillium expansum</i>	Synergistic action of thyme extract and chitosan provided broad spectrum control over fungi and certain bacteria by cellular leakage and essential metal ion chelation.	(54)
Chitosan-silver nanofilm	415–420	415–420	±50.08–±87.75	<i>Colletotrichum gloeosporioides</i>	Markedly reduced conidial germination, subcuticular mycelial growth and appressoria formation in ripening fruits.	(55)

involvement of RNA in the transfer of genetic information to all organisms. This led to the development of RNAi-based disease control strategies against several plant pathogens (63). Such RNAi-based gene silencing can be achieved through the transgenic approaches by delivering dsRNAs, siRNAs or miRNAs via vectors like *Agrobacterium tumefaciens*. However, strict regulations on genetically modified (GM) crops have shifted focus towards exogenous application of dsRNA through foliar sprays, trunk injection or root application. As naked dsRNA is prone to harsh environmental conditions, nanocarriers like chitosan are employed for their safe delivery. Under mildly acidic conditions, the amino groups of chitosan electrostatically interact with the negatively charged phosphate backbone of RNA, enabling successful encapsulation of the nucleic acid (64).

For instance, disease suppression using chitosan-dsRNA nanocomposites has been demonstrated against *R. solani* in tobacco by ϵ -poly-L-lysine and carboxymethyl chitosan-dsRNA nanoparticles targeting cell wall-degrading genes (65). In rice, chitosan-based polycation-loaded dsRNA complexes provided up to 20 days of resistance against the same pathogen and outperformed other carriers such as polyethylenimine, protamine, carbon quantum dot and polyamidoamine (66). Further studies show that dsRNA delivery efficiency depends upon the methods of synthesis of such carrier molecules. Nanoparticles prepared by the filtration method proved to be more efficient in controlling *Botrytis cinerea* upon foliar application (67). These findings highlight the role of chitosan not only as a protective matrix, but also as an enhancer of dsRNA stability, uptake and persistence. Though current findings demonstrate notable control of plant diseases, dsRNA remains highly prone to degradation under adverse environmental conditions. Therefore, proper encapsulation within chitosan matrices is essential to enhance dsRNA delivery (Fig. 2).

Mode of action of chitosan-based nanoformulations

Chitosan-based nanoformulations act against pathogens through multiple mechanisms, including membrane disruption, reactive oxygen species (ROS) generation, nutrient chelation, elicitation of host defence pathways and electrostatic interaction between the cationic groups of chitosan and anionic membranes of microbes (26). They are found to have a dual mode of action, working as a direct antimicrobial agent, as well as a defence elicitor (Fig. 3).

Direct antimicrobial action

Mode of action against fungi

In fungi, chitosan-based nanoformulations inhibit mycelial growth and sporulation by forming thick coatings on the fungal cell surface, which induce hyphal malformation and cell death. They also

enhance the beta-1,3-glucanase activity promoting cell wall degradation and membrane permeabilisation (68). Following penetration into the cell, chitosan interacts with the nucleic acids by electrostatic interaction with the negatively charged phosphate backbone of the nucleic acids (69). This hinders the normal translation and transcription processes, preventing the formation of infectious proteins. Ionic imbalance of calcium and potassium ions after treatment further results in the leakage of nucleotides, phosphates and enzymes required for fungal differentiation (70). However, those fungi with a higher proportion of polyunsaturated fatty acids in their plasma membrane would be more sensitive to chitosan due to the increased membrane fluidity. Conversely, those rich in saturated fatty acids would be less susceptible to the action of chitosan (71).

Mode of action against bacteria

Chitosan-based nanoformulations exhibit greater inhibitory activity against fungi. Studies have also confirmed their significant antibacterial potential against economically important target pathogens such as *Xanthomonas* sp., *Pseudomonas syringae*, *Ralstonia solanacearum*, *Clavibacter michiganensis* and other related pathogens. Chitosan nanocomposites penetrate bacterial cells and interfere with mRNA synthesis, disrupting the normal gene expression (72). The metal chelation ability of chitosan further deprives bacteria of their essential nutrients (11). Chitosan-zinc oxide nanoparticles were found to coat the bacterial cells when used against *Xanthomonas oryzae*, effectively entrapping the cells and reducing bacterial motility by about 30 % (73). Certain formulations have also been shown to disrupt quorum sensing mechanism, biofilm formation and swarming, thereby interfering with bacterial virulence (74). Owing to these properties, chitosan nanoformulations can be used as low-risk alternatives to conventional antibiotics.

Mode of action against viruses

Chitosan-based nanoformulations form protective films on the aerial surfaces of plants, acting as a physical barrier to viral entry. Within plant cells, chitosan regulates the synthesis of RNA-dependent RNA polymerase (RdRP), contributing to viral inactivation by RNA interference (RNAi) (75). Chitosan can function as a carrier for dsRNA, which, after entry into the target cells, activates silencing of virulence genes (67). Viral suppression can also be achieved by incorporating other virucidal substances like metal particles, essential oils, plant extracts, or even certain chemicals along with chitosan in the form of nanoformulations. Chitosan-dextran nanocomposites have been used to target the management of alfalfa mosaic virus in *Nicotiana glutinosa* L. plants. They observed that pre-inoculation foliar sprays of these composites led to a 70 % reduction in virus accumulation and post-inoculation

Table 4. Chitosan as a nano-carrier for biocontrol agents

Biocontrol agent	Particle size (nm)	Pathogen inhibited	Key findings	References
<i>Trichoderma asperellum</i>	-	<i>Phaeoconiella chlamydospora</i> and <i>Phaeoacremonium aleophilum</i>	Chitosan - <i>T. asperellum</i> based poly (L-lactic acid) (PLLA) nano-film reduced grapevine esca disease; less viscous chitosan allowed better embedding of <i>Trichoderma</i> spores.	(59)
<i>Streptomyces</i> sp. metabolites (toyocamycin, nystatin, anisomycin, natamycin)	60–200	<i>Colletotrichum gloeosporioides</i>	Reduced conidial germination and quiescent infection; enhanced foliar adhesion. Disease control efficacy was 72.97 %.	(60)
<i>Trichoderma harzianum</i>	28.59 ± 4.23	<i>Sclerotium rolfsii</i>	Copper-chitosan guar-gum nanocomposite encapsulating <i>Trichoderma harzianum</i> spores synergistically inhibited <i>S. rolfsii</i> ; increased shelf life and root colonisation.	(61)

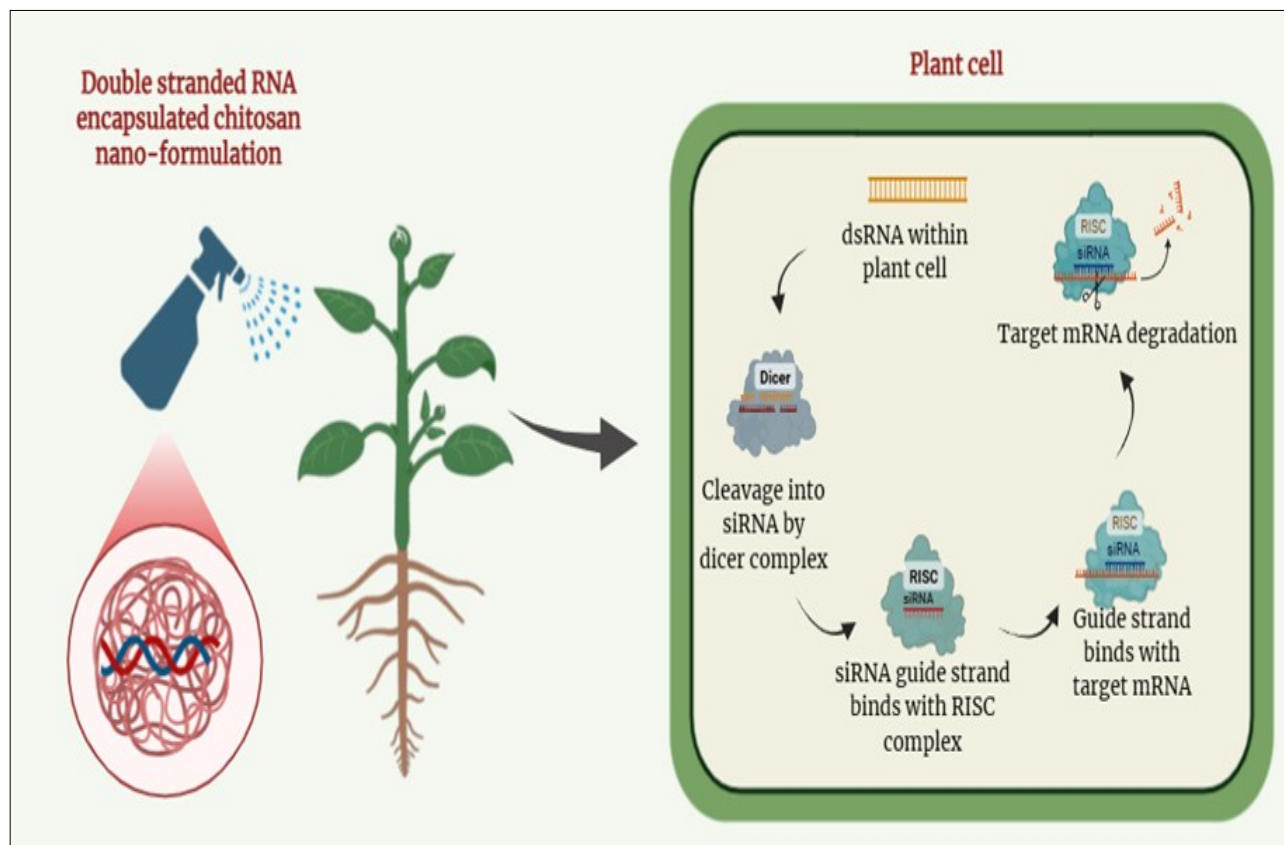


Fig. 2. Chitosan-mediated delivery of dsRNA for gene silencing; following exogenous application of the nanoformulation, dsRNA is cleaved into small interfering RNA (siRNA) and incorporated into RISC, leading to sequence-specific degradation of target mRNA.

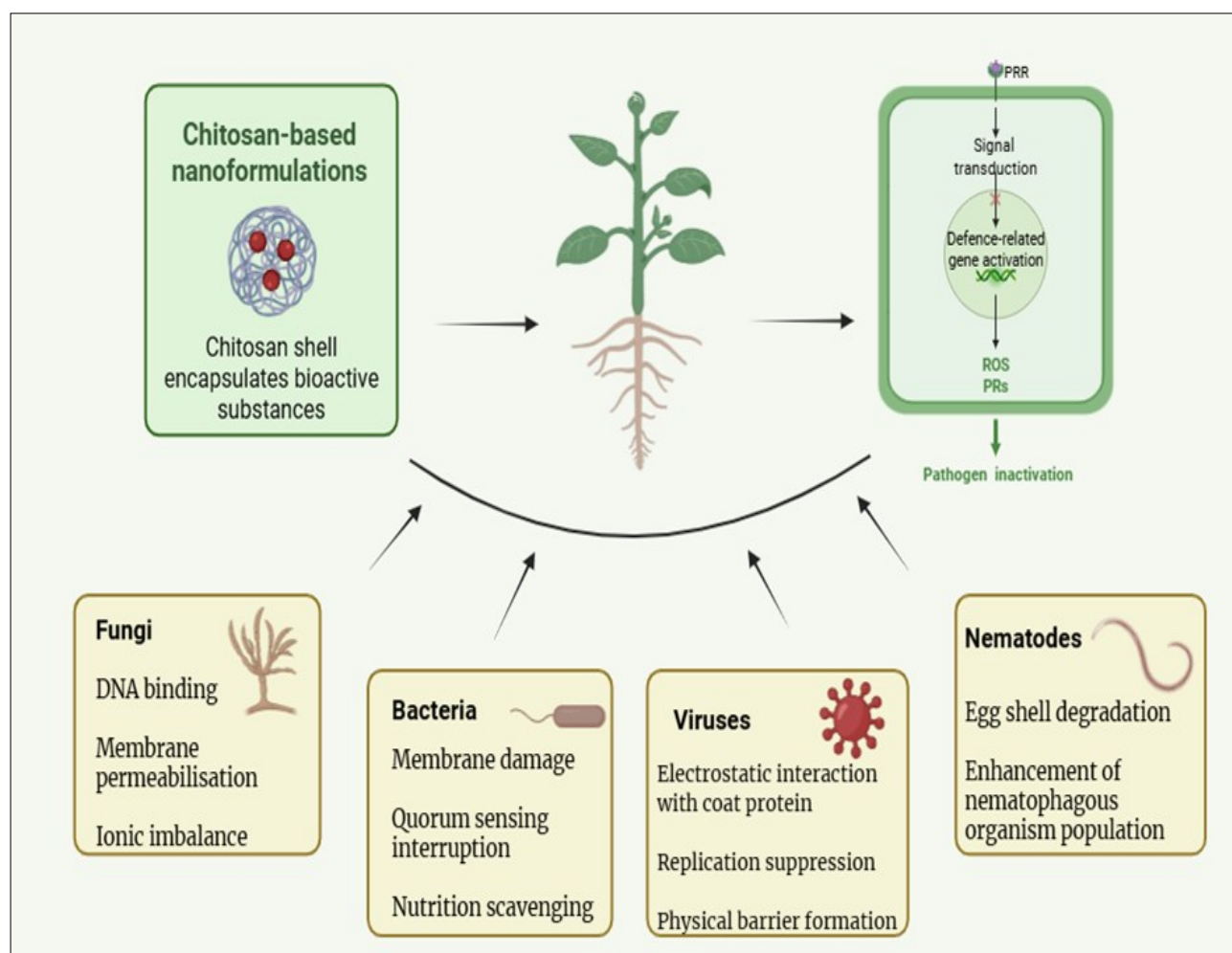


Fig. 3. Diverse modes of action of chitosan-based nanoformulations against fungi, bacteria, viruses and nematodes. PRR: pattern recognition receptors; ROS: reactive oxygen species; PRs: pathogenesis related.

sprays achieved about 61 % inhibition. The virucidal property of the formulations was tested by the 'inactivation method', also by mixing the viral suspension directly with chitosan nanocomposite, resulting in complete suppression of infection (76). However, only limited evidence exists for the direct control of viral pathogens using only chitosan. In most cases, viral inhibition occurs primarily through host defence activation mediated by the biopolymer.

Against nematodes

Chitosan-based nanoformulations offer promising nematocidal activity through multiple mechanisms. Such activity can occur through direct toxicity, induction of chitinolytic enzymes and enhanced delivery, of nematocides using chitosan as a carrier. Soil amendment with chitosan stimulates beneficial soil microbes to produce chitinolytic enzymes. These enzymes degrade nematode eggshells and juvenile cuticles, by breaking down chitin into chitobiose. In *Meloidogyne incognita*, chitosan nanoparticles at 2000 ppm resulted in 97.5 % juvenile mortality and 95.3 % inhibition of egg hatching (77). Greenhouse studies revealed a significant decrease in overall root gall formation at all concentrations of the nanoformulation, accompanied by a significant increase in shoot and root length (78). Although chitosan-based nanoformulations broadly suppress fungal pathogens, they selectively promote the growth of certain beneficial fungi such as *Pochonia* sp., which are parasitic to nematodes (79).

Indirect antimicrobial action

Plant defence induction

Besides direct antimicrobial action, chitosan functions as a defence elicitor by activating systemic acquired resistance (SAR) and inducing systemic resistance (ISR). It mimics pathogen-associated molecular patterns (PAMPs) and is sensed by the membrane-bound pattern recognition receptors (PRR). Some evidence suggests that the chitin elicitor receptor kinase (CERK1) binds to chitosan, triggering downstream signalling pathways such as mitogen-activated protein kinase (MAPK) and receptor-like kinase (RLK) pathways within the plant. These pathways activate several transcription factors and defence-related genes, thereby priming the plant against future infections (80). After signal perception, plants respond via oxidative burst at the site of infection, producing reactive oxygen species, primarily hydrogen peroxide (H_2O_2) at the site of infection. Reactive oxygen species function as direct antimicrobial molecules and defence elicitors. This has been documented in plants like *Capsicum annuum* L., *Pinus pinaster* Aiton. and *Melissa officinalis* L. (81).

Chitosan-based treatments have been shown to upregulate the production of pathogenesis-related (PR) proteins and defence enzymes. Chitinases and β -1,3-glucanases are associated with fungal cell wall degradation; peroxidases and polyphenol oxidases are responsible for ROS generation and phenylalanine ammonia-lyase (PAL) induces the accumulation of phytoalexins and lignin (29, 82). For instance, in crops like maize and tomato, chitosan nanocomposites elevated defence enzyme activity, promoted callose and lignin deposition and significantly reduced pathogen invasion (83). At low concentrations, these formulations trigger calcium ion efflux- the primary signal transducers in the plant cytosol, resulting in programmed cell death by cytoplasmic shrinkage, chromatin condensation and activation of caspase-like protease. This hypersensitive response blocks further pathogen propagation (84). When applied as a pre-treatment, or

during the initial phases of infection, chitosan nanoformulations act as effective tools for significantly reducing disease incidence in plants.

Release kinetics of chitosan-based nanoformulations

Chitosan-based nanoformulations regulate the slow and targeted release of encapsulated bioactive substances. The release of these compounds from chitosan matrices predominantly occurs through matrix swelling, diffusion of active ingredients and matrix erosion (85). In most nano-chitosan systems, these mechanisms operate simultaneously resulting in efficient and sustained active ingredient transport.

Swelling-controlled release is one of the primary mechanisms controlling release kinetics in chitosan-based nanoformulations. Owing to its hydrophilic nature, the exposure of chitosan nanoparticles to moist environments enables water absorption and consequent matrix swelling (86). This creates additional pathways within the polymer network for the gradual diffusion of encapsulated compounds. However, the extent of swelling of chitosan is greatly influenced by the degree of deacetylation, molecular weight and extent of cross-linking (87). Diffusion-controlled release occurs when the encapsulated biomolecules migrate through the swollen chitosan matrix into the surrounding medium. Either Fickian or non-Fickian diffusion might occur, depending on the relative rates of solvent diffusion and polymer relaxation. Densely cross-linked networks result in slower release profiles (85). Erosion or degradation-controlled release takes place when the chitosan shell is structurally disrupted by environmental factors such as hydrolysis, enzymatic degradation or mechanical disruption in the soil environment. As the polymer network dismantles, the entrapped agents are released steadily (88) (Fig. 4).

Chitosan-based nanoformulations are notable for their pH-responsive release behaviour as well. This is due to protonation and deprotonation of amino groups, which alter the polymer swelling and relaxation. Under acidic conditions, protonation increases electrostatic repulsion within the matrix, resulting in the burst release of active ingredients (87). For example, nanochitosan loaded with kasugamycin has been reported to release nearly 50 % of the encapsulated kasugamycin per hour at low pH, whereas neutral to alkaline conditions favoured a more sustained release (89). Such nanoformulations offer a comparative advantage in plant disease control as certain pathogens create acidic environments at infection sites through the secretion of organic acids (90). At neutral to alkaline conditions, protonation is reduced, which limits swelling, resulting in a more sustained release. The responsiveness of the chitosan-based nanoformulations can be improved with the addition of pH-sensitive polymer grafts, such as polyacrylic acid (PAA). Such inter-polymer complexes enable non-Fickian controlled release of the agro inputs by influencing the swelling behaviour (91). Additionally, ionic strength also modulates release kinetics by affecting interactions within the chitosan matrix, with higher salt concentrations resulting in release through salting-in effects (92). However, this stimuli-responsiveness of chitosan-based nanoformulations is influenced by the physicochemical properties of the polymer.

Entry, uptake and translocation of chitosan-based nanoformulations in plants

The efficiency of chitosan-based nanoformulations is influenced by

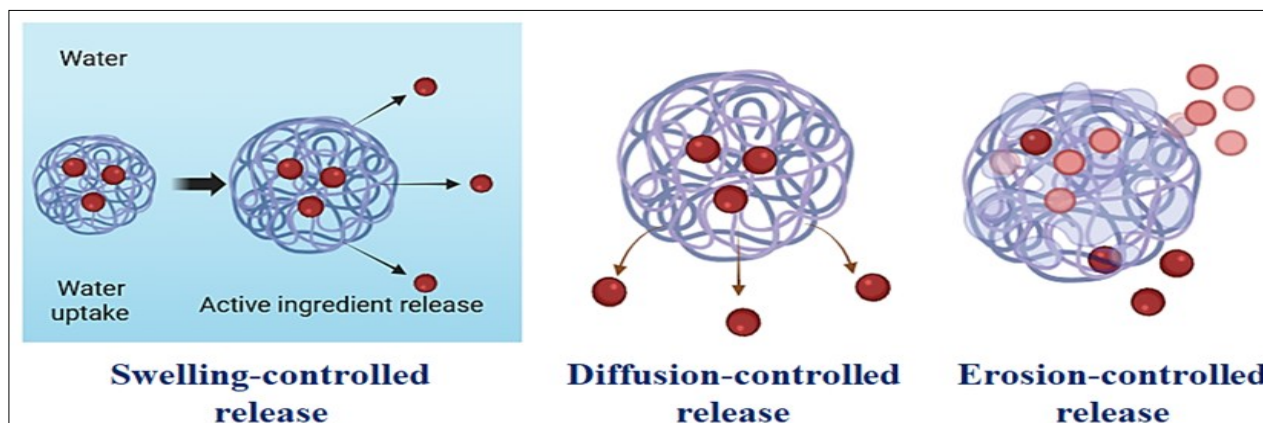


Fig. 4. Mechanisms for release of active ingredients from chitosan nano-carriers.

their ability to penetrate plant tissues and move within the plant system. Proper uptake and movement of the formulation depend on nanoparticle size, shape, charge and surface chemistry, which influence the efficient delivery of active substances to infected sites. Nanoparticles enter the plant system through aerial or below-ground pathways (93). During entry, they must overcome the plant's natural barriers, primarily the cuticle, cell wall and cell membrane. Usually, this process is mediated by pathways such as diffusion, endocytosis or direct uptake via membrane-associated transport channels (94). Upon foliar application, entry usually occurs primarily through stomata and hydathodes, which offer significantly large pore size compared to the cuticle. Moreover, the ability to traverse the cuticle depends on nanoparticle adhesion and hydrophobicity. After penetration, the particles move through intercellular spaces via apoplastic or symplastic routes within the plants (93). The root uptake of nanoparticles is strongly influenced by surface charge. Root surfaces carry an overall negative charge which attracts positively charged particles to aggregate at the epidermis, restricting further penetration. On the other hand, negatively charged or neutral particles show less interaction with the root surface, facilitating unimpeded penetration and translocation via the vascular bundle (95). Furthermore, smaller nanoparticles can traverse the root epidermis and cortex with greater ease, while larger particles are retained at the surface. Internal structures such as the casparian strip act as selective barriers restricting further movement into the xylem (96). Other external factors like ionic strength, pH and root exudates can further influence nanoformulation stability by promoting aggregation, thereby altering nanoformulation bioavailability (97). Optimally sized polymeric nano-carriers themselves can be readily internalised and transported via apoplastic pathways, enabling carrier-mediated co-transport of active ingredients (98). Understanding these processes and transport mechanisms is essential for designing nanoformulation with optimised bioavailability and targeted delivery efficacy.

Ecological safety and fate after application of chitosan-based nanoformulations

Chitosan, owing to its natural origin and biodegradability, is categorised as 'Generally Recognized As Safe' (GRAS) for use in food and agriculture (99). The United States (US) Environmental Protection Agency (EPA) has classified chitosan under minimum risk pesticide products, underscoring its biosafety (100). Following soil application, it undergoes gradual depolymerisation mediated by chitosanases and lysozymes secreted by soil microbes. The breakdown products of chitosan include glucosamine, various oligosaccharide intermediates, carbon dioxide and water which enter into natural biogeochemical cycles, thereby minimising their

accumulation (101, 102). Thus, chitosan-based nanomaterials often exhibit improved biodegradability compared with many inorganic nanomaterials. Acute toxicity studies of chitosan in mammals have revealed high LD_{50} values indicating minimum safety concern (103). In pollinators, supplementation with low concentrations of chitosan nanoparticles improved the colony performance without inducing toxicity (104). Studies on earthworms also demonstrated that these nanocomposites exhibit lower mortality rate compared to conventional pesticides (105). Similarly, current studies indicate that chitosan does not significantly affect the proliferation of beneficial soil microbiota or their colonisation of plant roots. These observations suggest that, despite its antimicrobial activity, chitosan applied within recommended agronomic limits is beneficial to plant health without disrupting soil ecology (106). Nevertheless, most of these studies have been conducted under controlled conditions and over short periods of exposure. The effect of repeated applications of chitosan-based nanoformulations on the beneficial microbes and non-target organisms remains less understood. Overall, polymeric nanocarriers like chitosan are considered safer than raw metallic or carbon-based nanomaterials, provided their concentration remains below the lethal dose.

Economic feasibility of chitosan-based nanoformulations

The translation of chitosan-based nanoformulations from experimental systems to field conditions greatly depends on their cost-effectiveness compared with commercial pesticides. The economic feasibility of chitosan-based formulations is influenced by the raw material availability and the production cost of chitosan, the additional investment required for its conversion into nanoformulations as well as the market cost of conventional fungicides. Chitosan is primarily produced by the deacetylation of chitin, extracted from the exoskeleton of crustaceans, which are available in large quantities as by-products of the global seafood industry (9). This marine waste can be procured approximately at a cost around USD 1920/tonne and the production cost of chitosan ranges from USD 8.39/kg in abundant shrimp-producing regions such as Ecuador to approximately USD 14/kg in regions with limited crustacean by-product availability (107). Establishing chitosan production units adjacent to seafood processing facilities, combined with large-scale batch production can further improve economic viability. At the commercial level, agriculture-grade chitosan is priced between USD 10 and USD 1000/kg, depending on purity and application (10). Though the conversion of chitosan into nanoformulations may involve expensive technologies, they offer advantages beyond the initial purchase price. Despite the lower cost of synthetic fungicides, they carry significant indirect expenditures related to resistance management, residue monitoring and

regulatory issues (4). Chitosan nanoformulations offer multiple agronomic benefits within a single application, functioning simultaneously as antifungal agents, plant defence elicitors and growth promoters (7).

The growing intellectual property landscape further reflects the potential for translating experimental chitosan nanoformulations into commercial crop protection products. Certain patents have been granted in this field, such as ES1304317U which describes chitosan nanoparticles encapsulating garlic essential oil exhibiting antifungal potential against *Aspergillus* sp. and *Fusarium* sp. (108). Similarly, patent number US10172353B2 describes a chitosan-based fungicide formulation for plant disease control (109). However, the limited progression of experimental formulations and filed patents into registered products highlights the persistent gap between research and marketing.

Global regulatory landscape

The commercialisation and deployment of chitosan-based formulations in agriculture requires regulatory approval which varies across countries. Synthetic fungicides have a standardised registration process unlike biopolymer-based nanoformulations which lack clarity. Chitosan-based nanoformulations are often regarded as biopesticides, biostimulants or minimum risk pesticides in different regions. This fragmented classification structure creates uncertainty for researchers to scale up the commercialisation of such products. An overview of regulatory frameworks of different countries has been listed in Table 5.

Conclusion

Chitosan-based nanoformulations offer an eco-friendly and effective approach to plant disease management. They are non-toxic and biodegradable and provide broad-spectrum control against diverse pathogens, including fungi, bacteria, viruses and nematodes. These formulations leverage the inherent biocidal activity of chitosan, along with the potential benefits of nanotechnology, thus making them versatile platforms for plant disease management. Chitosan's cationic property and mucoadhesive nature enable these

formulations to bind with pathogenic membranes, initiating their destruction and gradually leading to their death. Despite exhibiting direct antimicrobial activities against the notorious plant parasites, these agro-nanoformulations work in a dual manner by eliciting the upregulation of defensive genes and proteins within the plants and by promoting plant growth. Even though chitosan-based nanoformulations are extensively advantageous, their large-scale adoption faces certain constraints related to stability, environmental exposure and safety that need to be addressed. Inadequate surface charge may cause particle aggregation, leading to increased particle size and reduced stability during storage. Furthermore, the premature release of encapsulated compounds under unfavourable pH conditions may reduce their performance in field applications. Therefore, future research should focus on the standardisation of formulation protocols, development of highly stable nanoformulations and comprehensive toxicity evaluation using advanced analytical and risk-assessment tools to support their safe and effective commercialisation. Advanced analytical techniques such as high-performance liquid chromatography-mass spectrometry (HPLC-MS), liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) should be employed for degradation profiling and nanoparticle persistence studies. Additionally, reliable data on product stability, residual analysis and environmental fate should be generated for meeting regulatory requirements. The integration of chitosan-based nanoformulations with precision farming technologies, including sensor-based disease monitoring and triggered release systems can further support sustainable crop protection strategies.

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Table 5. An overview of the global regulatory landscape of chitosan-based products

Country	Regulatory body/key legislation	Key legislation	Classification of chitosan	Approval status	References
United States of America	Environmental Protection Agency (EPA)	FIFRA Section 25 (b); 40 CFR 152.25 f	Minimum risk pesticide	Exempted from FIFRA registration; effective from January 9, 2023	(110)
European Union	European Food Safety Association (EFSA)	Article 23 of Regulation (EC) No. 1107/2009; Article 29 of Regulation (EC) No. 178/2002	Basic substance (chitosan hydrochloride); plant protection agent and elicitor	Approved for chitosan (2022) and chitosan hydrochloride (2014)	(111)
India	Central Insecticides Board and Registration Committee	Insecticides Act, 1968	Biostimulant	No dedicated approval or regulation for chitosan-based products; it can be registered under biopesticides	(112)
China	Ministry of Agriculture and Rural Affairs (MARA)	-	Biopesticide and plant elicitor	Several chitosan oligosaccharide-based products have been registered	(113)
Brazil	Ministry of Agriculture, Livestock and Food Supply (MAPA)	-	Biochemical biopesticide	No dedicated approval or regulation for chitosan-based products	(114)

Authors' contributions

BPN performed literature collection and prepared the original manuscript. JI conducted supervision, formal analysis and validation of the manuscript. KM participated in the supervision and conceptualisation of the manuscript. AS assisted in language editing. RK and CK contributed to conceptualisation. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly in order to improve language and readability, with caution. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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