

BIOPESTICIDE APPLICATIONS OF CHITOSAN-BASED NANOMATERIALS: HUGE POTENTIAL AND INNOVATION STRATEGY FOR MODERN AND SUSTAINABLE AGRICULTURE IN VIETNAM

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Abstract

Agriculture is a backbone of global economy and of Vietnam in particular, providing food, fiber, and numerous other products to sustain human life. Chitosan has emerged as a non-toxic, biodegradable, and biocompatible natural biopolymer with multiple beneficial applications in the fields of agricultural and biomedical sectors. As nanotechnology has evolved as a promising field, researchers have applied chitosan-based nanomaterials in a variety of products. Due to its inherent antimicrobial and chelating properties, and the availability of modifiable functional groups, chitosan has been used as an encapsulating agent for bioactive plant compounds and agrochemicals by different technologies, such as ionotropic gelation, spray-drying, nanoemulsions, coacervation, reverse micelles, and sieving method to enhance antimicrobial activity, efficacy and biocompatibility. Chitosan-based nanomaterials have been shown to increase potential antibacterial, and antifungal activity against pathogens, presenting higher stability, decreasing degradation, and prolonging the effective concentration of these bioactive compounds. Therefore, the objective of this work is to review the most recent developments

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in the synthesis of chitosan- based nanomaterials as well as the applications on phytopathogenic diseases. These perspectives could provide information for the future formulation of products with high efficacy against phytopathogenic diseases as an alternative to chemical products for sustainable and modern-day Vietnam agriculture.

1 Introduction

Recently, the quest for sustainable agricultural practices has gained significant attention, driven by the need to ensure food security, reduce environmental impact and enhance crop productivity. Vietnam has set an objective to build a modern and sustainable agriculture economy during the 2021-2030 periods, with a vision to 2050. Sustainable development regarding safely reusing waste and the transformation of waste to valuable products is emerging as a strong trend.

In agriculture, plants are susceptible to environmental damage and pathogen attack, which causes crop diseases and economic losses. Therefore, different strategies have been developed to reduce plant diseases caused by pathogens and nutrients such as fertilizer, pesticides while minimizing the environmental impact [1-10]. In this sense, biopesticides have received great attention from scientists as promising alternatives to chemical pesticides mainly because they comprised of plant-derived active ingredients to control pests and pathogens [11,12].

Chitosan is carbohydrate biopolymer that can be isolated from marine waste and microorganisms; this biopolymer is widely studied for high biocompatibility, biodegradability, low toxicity, film-forming ability, antimicrobial activity, and specific interactions with phytopathogenic microorganisms and for improving the productivity of different crops [13-17]. In this sense, chitosan has demonstrated an effect on crop productivity and potential protection against phytopathogenic agents such as fungi and bacteria [18,19]. For agriculture application purposes, chitosan must be soluble in neutral aqueous solutions, thermodynamically stable, and have a small particle size. Nanotechnology can play an essential role in addressing these issues. The small size of the nanomaterials is advantageous in crossing the biological barriers and allowing for increased contact between the inputs and the plant, thus improving the efficacy and reducing the amount of input needed [1,2, 20-23]. In this sense, chitosan-based nanomaterials (ChNPs) have been obtained by different techniques such as ionotropic gelation, spray-drying, emulsion crosslinking, coacervation technique, reverse micelles, and sieving method to encapsulate bioactive compounds on crops to enhance and protect against fungi and bacteria [12,24-27]. Therefore, this manuscript aims to review the current state of knowledge of the potential effect of ChNPs with different techniques on inhibiting phytopathogenic

microorganisms and crop production.

Definition, sources of chitosan and chitosan-based nanomaterials

After cellulose, chitin is the major natural polymer in the world [24]. Chitin is sourced from various origins, including the shells of sea animals such as crustaceans, annelida, coelenterate, lobster, shrimp, prawn, mollusks, crabs, and krill. Additionally, chitin can be extracted from insects like brachiopods, from microorganisms such as yeast, green algae, fungal cell walls, brown algae, *Penicillium* mycelia, chytidiceae, blastocladiacea, and spores [28,29]. Chitin, a cellulose-like polysaccharide, is a linear, *poly* – β – (1,4) – *N* – acetyl – *D* glucosamine [30]. Chitosan, also known as deacetylated chitin, is a naturally occurring polycationic polysaccharide derived from partial deacetylation of chitin (as shown in Figure 1). It is a linear copolymer with D-glucosamine and N-acetyl-D-glucosamine units joined via β – (1 – 4) glycosidic bonds.

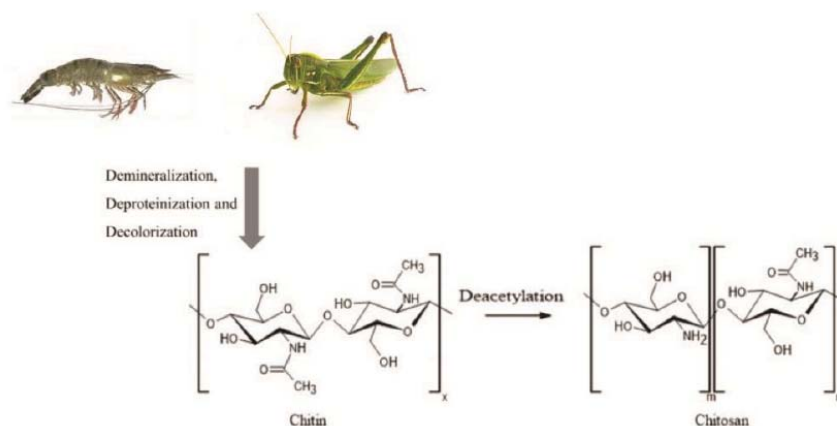


Figure 1. Process of chitosan production starting with different sources.

Chitosan is insoluble in water, organic solvents, and aqueous bases and it is soluble in acids such as acetic, nitric, hydrochloric, perchloric and phosphoric [31]. Chitosan has been popular due to its antimicrobial, antioxidant, and chelating properties, together with its nontoxic and biocompatible nature [18]. As a cationic polymer, chitosan inherently possesses bio-adhesion, cellular transfection, anti-inflammatory, and anti-hypercholesterolemic characteristics, which can be enhanced by combining with other materials, making it an attractive candidate for agricultural applications [15]. The availability of hydroxyl and amino groups on chitosan provides an excellent platform for complexation with other molecules/compounds and helps to transform them into more stable complexes [32]. Additionally, due to the availability of func-

tional groups, chitosan can be modified in different ways to obtain substituted, crosslinked, carboxylated, ionic and bounded derivatives to match the various research needs [33].

Nanoparticles are a type of colloidal system comprising particles with a size range from 10 to 1000 nm in diameter ($1\text{nm} = 10^{-9}\text{m}$) [34]. Nanoparticles exhibit size-related physiochemical properties that make significantly differ from those observed in fine particles or bulk materials [35]. In agriculture sector, chitosan nanoparticles by themselves can be used as pesticides, herbicides, insecticides [36]. ChNPs enhance the bioactive components' dissolution, entrapment, encapsulation, and adhesion capability to the nanoparticle matrix [37]. These structures possess large surface areas that facilitate the absorption of bioactive agents. Additionally, their tiny nanoscale dimensions enhance the effective penetration through cell membranes. Specially, when it comes to plant cells, nanoparticles with a size smaller than 20 nm in at least one dimension possess the ability to effortlessly enter [38]. Significant difference in the activity of encapsulated material in chitosan nanoparticles was observed as compared the same non-capsulated material [24].

Synthesis of chitosan nanoparticles

Various methods were adopted to prepare nanosize material from chitosan including ionic gelation, spray drying, emulsion crosslinking, coacervation technique, reverse micelles, and sieving method [24]. Generally, the most frequently deployed techniques for the large-scale production of chitosan-based nanomaterials are ionotropic gelation and spray drying.

Ionotropic gelation

Ionotropic gelation is a commonly used method for producing chitosan-based materials due to its simplicity and better control over particle size and distribution [39]. In the first stage, chitosan is dissolved in an acidic solution, typically acetic acid, to form a chitosan solution wherein the chitosan concentration varies depending on the desired nanoparticle properties. The second stage is preparing polyanion solution, which is prepared separately; commonly used polyanions are sodium triphosphate (TPP) and sodium sulfate. Next, the chitosan and the polyanion solution are mixed under constant stirring. As they come into contact, the positively charged amino groups of chitosan interact with the negatively charged polyanions, forming a gel-like suspension of ChNPs comprising the crosslinking of chitosan chains. To enhance the stability and prevent aggregation of the nanoparticles, additional steps may be included involving pH adjustment, centrifugation, and the addition of stabilizing agents such as surfactants. Finally, the ensued ChNPs are typically washed to remove unreacted chitosan or polyanion residues. They are usually freeze-dried or lyophilized to achieve a dry powder form that can be stored for further

usage [40,41]. The ionotropic gelation method is the most straightforward and economically viable approach for scaling up from laboratory to industrial production. This superiority stems from its reliance on uncomplicated and affordable materials and equipment, allowing for its relatively swift and feasible implementation within conventional research laboratories [42]. Moreover, the underlying principle of electrostatic interaction, as opposed to a chemical reaction, obviates the necessity for using organic solvents, thereby mitigating potential toxicological risks. Additionally, when carefully established to facilitate optimized polymer-core agent interactions, this technique can potentially enhance encapsulation efficiency [43-46].

Spray drying

Spray-drying transforms a fluid (emulsion, dispersion, solution) into dry particles by spraying the solution in a heated air stream. This technique allows for the formation of ChNPs with controlled particle size and morphology [47]. Initially, a chitosan solution is prepared by dissolving chitosan in an appropriate solvent, usually acetic acid, or hydrochloric acid where its concentration can vary depending on the desired nanoparticle characteristics. A surfactant or stabilizer may be added to the chitosan solution to realize uniform dispersion of chitosan particles, which helps prevent particle aggregation during spray drying. The chitosan solution is then atomized into small droplets using an atomizer. Various types of atomizers are deployable, such as pressure nozzles, rotary atomizers, or ultrasonic atomizers and their choice depends on factors like desired particle size and production scale. The atomized droplets are introduced into a drying chamber or tower, where they come into contact with a stream of hot air or gas. The heat causes rapid evaporation of the solvent, leaving behind solid ChNPs. The dried ChNPs are collected from the bottom of the drying chamber. Depending on the specific requirements, additional post-processing steps such as sieving or milling might be carried out to attain particles of desired size distribution [48]. Overall, spray drying offers a versatile and scalable method for producing CNPs with controlled particle size and improved stability, making them suitable for diverse applications in various industries.

Emulsion crosslinking

This technique involves the formation of a nanoemulsion followed by crosslinking of chitosan to stabilize the nanoparticles [49]. Nanoemulsions are colloidal (lipidic) systems in which two immiscible liquids are dispersed with each other. One of the liquids is the solvent, while the other is the dispersed phase. As usual, a chitosan solution is prepared initially by dissolving chitosan in an acidic solution, the concentration being adjusted according to the desired nanoparticle characteristics. An organic phase is created in a separate container by dissolving a crosslinking agent (such as glutaraldehyde or genipin) in an or-

ganic solvent (like dichloromethane or chloroform). Subsequently, the chitosan solution is added dropwise into the organic phase while vigorously stirring to form an emulsion consisting of small chitosan solution dispersed in the organic phase. An emulsifying agent may be added to stabilize the emulsion and prevent coalescence of droplets. After emulsion formation, the crosslinking agent is introduced into the emulsion. The emulsion is then stirred for a specific duration to facilitate crosslinking and curing of the nanoparticles. To extract the nanoparticles from the organic phase, an aqueous phase (usually water) is added, which causes the nanoparticles to migrate from the organic phase into the water phase. The nanoparticles are collected by filtration or centrifugation, followed by washing with a suitable solvent (such as water) to remove any impurities or residual chemicals. Finally, the ChNPs are dried using freeze-drying or vacuum-drying techniques to obtain a dry powder form that can be stored or used in further applications [50].

Coacervation technique

Coacervation technique involves the phase separation of two oppositely charged polymers or a polymer and a non-polymeric material [51]. In the case of ChNPs, chitosan is typically combined with an oppositely charged polymer, such as sodium alginate or gelatin, in a solvent system where they do not mix thoroughly. This combination leads to the formation of coacervates, which are liquid droplets rich in the polymer mixture. By further processing, such as crosslinking or solvent evaporation, these coacervate droplets can be transformed into solid ChNPs [52].

Reverse micelles

To prepare ChNPs using reverse micelles, a combination of hydrophobic and hydrophilic surfactants like Span 80, Tween 80, or sodium bis(2-ethylhexyl) sulfosuccinate (AOT) is often deployed. These surfactants self-assemble in an organic solvent, such as ethanol or chloroform, forming reverse micelles. The next step entails the preparation of the oil phase by dissolving chitosan in organic solvents and surfactants. Then, an aqueous solution is prepared separately using water or an aqueous buffer. The oil phase, containing dissolved chitosan, is slowly introduced into the aqueous phase while stirring or sonication is applied. As the oil phase merges with the aqueous phase, chitosan molecules become encapsulated within the reverse micelles, forming ChNPs. Subsequently, the organic solvent evaporates under reduced pressure or other suitable methods, leaving behind the ChNPs. To ensure purity, the nanoparticles can be further purified through centrifugation or filtration, removing the excess surfactants, organic solvents, and oversized particles [53]. The specific details of the reverse micelle method may vary depending on factors such as the desired nanoparticle size, chitosan concentration, choice of surfactants, and other experimental conditions [54].

Sieving method

The sieving method is a widely used technique for producing ChNPs involving several steps. Firstly, chitosan is dissolved in an acidic solution to form a chitosan solution. Next, the chitosan solution is poured onto a sieve or filter with a specific pore size which determines the size of the nanoparticles that will be formed. The chitosan solution is then subjected to filtration under controlled pressure or vacuum, allowing the solvent (usually water) to pass through the sieve while retaining the ChNPs on the surface. As the solvent continues to pass through the sieve, the chitosan molecules start to aggregate and form nanoparticles due to the decreased solubility and enhanced intermolecular interactions. These nanoparticles are trapped on the sieve due to their larger size, while smaller molecules and impurities pass through. After filtration, the ChNPs are carefully collected from the sieve surface, further washed and purified to remove any residual impurities or solvent. Various characterization techniques like electron microscopy, dynamic light scattering, and zeta potential analysis can be employed to assess the size, morphology, and stability of the generated CNPs [55]. In summary, the sieving method is a valuable tactic for producing ChNPs wherein controlling the chitosan solution concentration and deploying sieves with specific pore sizes allows for generating nanoparticles with desired properties for various biomedical applications.

Chitosan-based nanomaterials utilization as biopesticides

In the agriculture sector, ChNPs by themselves could act as an antimicrobial agent against the crop pathogenic microorganisms like fungi for eg. *Pyricularia grisea*, *Alternaria solani*, and *Fusarium oxysporum* and bacteria like gram positive and gram negative, and other insect pests like *Aphis gossypii*, *Callosobruchus chinensis*, and *Callosobruchus maculatus* and as a plant growth promoter [56,57].

Various essential oils encapsulated with chitosan have been tested with different phytopathogenic fungi, among which we find genera of important crops such as *Alternaria*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizoctonia*, and *Sclerotinia* [58-66]. Used of *Cuminum cyminum* essential oil loaded chitosan nanoparticles on the shelf life of button mushroom showed effective in maintaining color, firmness, and overall acceptability and inhibiting the investigated bacteria and mold and yeast growth [67]. In the vast majority, the essential oils themselves have a fungicidal effect. However, the encapsulation of these oils with chitosan greatly enhances the fungicidal effect due to the high stability that gives them in the chitosan polymer matrix, being its slower volatilization and/or degradation, prolonging the concentration that is effective in stopping the development of fungi [24].

The bactericidal efficacy of chitosan depends, in addition to the character-

istics of chitosan, on other microbial and environmental factors, such as the bacterial species, temperature, pH, and ionic power of the medium. Depending on the species, the minimum concentrations inhibitory to the growth of bacteria vary between 10 to 1000 ppm [12]. Based on available evidence, chitosan also prevents the growth of various plant pathogenic bacteria such as *Xanthomonas*, *Pseudomonas syringae*, *Agrobacterium tumefaciens*, and *Erwinia carotovora* [68-70]. It has been determined that the polyphenols extracted from olive leaf, encapsulated in chitosan nanoparticles, have functioned as fungicidal agents when controlling *Verticillium* wilt in tomato plants [71]. Pomegranate (*Punica granatum L.*) peel extract has also been encapsulated, in which loading efficiencies between 26-70% and a particle size between 174-898 nm exhibited antimicrobial activity against gram-positive *S. aureus* [72]. The synthesis of nanoliposomes by micro fluidization of tea polyphenols, obtaining around 78% encapsulation efficiency, as well as the size of 67 nm, inhibited the growth of *S. aureus*, *E. coli*, *S. typhimurium*, and *L. monocytogenes* [73].

Future trends and perspectives

It estimates that, at present, around 1,000 metric tons of farmed shrimp per day is wasted in Vietnam in the form of heads, shells, and anything else not traditionally used in producing seafood products [74]. This represents a huge source of raw materials for chitin extraction and chitosan production. According to the research paper [75], chitin production requires 33 kg shrimp shells in wet weight per kg chitin and chitosan production requires 1.4 kg chitin per kg chitosan. If we can employ technology to turn tons of annual shrimp waste, it would yield billions of dollars with the present market price of chitosan.

It can be seen from the available literature that the ChNPs could be a versatile, biodegradable, and biocompatible, low toxicity, and easy degrading alternative to presently available chemical pesticide. ChNPs can be applied in various fields based on their properties such as antibacterial, antifungal, antiviral, antioxidant activity. But the thorough studies on product development and method optimization are required before commercial production. As ChNPs are discovered to have the ability to encapsulate the various agro-supplements, they could be employed for the controlled deliberations of fertilizers, pesticides, and plant growth promoters in agriculture fields. Consequently, ChNPs have a huge contribution to reducing fertilizers pollution, managing agricultural pests and pathogens in modern and sustainable agriculture.

Institute of Applied Science and Technology (I.A.S.T) hope to connect with like-minded strategic partners, locally and overseas to create value from shrimp waste through collaboration. I.A.S.T are looking forward to joint projects with them in the near future.

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