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

Nguyen Van Tam and **Phan Phuoc Hien***

Institute of Applied Science and Technology,
Van Lang University, Ho Chi Minh City, Vietnam
e-mail: hien.pp@vlu.edu.vn

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 iontropic 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

*corresponding author;
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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 iontropic 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, $poly-\beta-(1,4)-N-acetyl-D$ 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 $\beta-(1-4)$ glycosidic bonds.

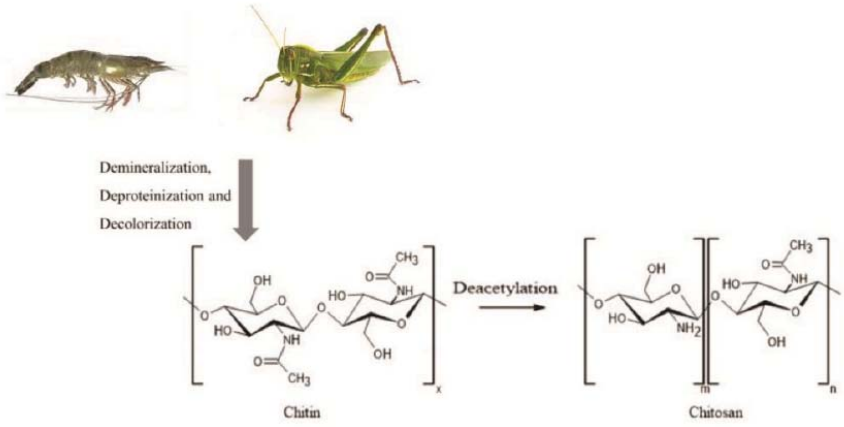

Figure 1. Process of chitosan production starting with different sources.

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 ($1nm = 10^{-9}m$) [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 crosslingking, 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.

Iontropic 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 tripolyphosphate (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 an nanoemulsion followed by crosslinking of chitosan to stabilize the nanoparticles [49]. Nanoemulsions are colloidal (lipidic) systems in which two immiscible liquids arevdispersed 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.

References

[1] [1] M. Hassanisaadi, A.H. Shahidi Bonjar, A. Rahdar, R.S. Varma, N. Ajalli, S. Pandey, *Eco-friendly biosynthesis of silver nanoparticles using Aloysia citrodora leaf extract and evaluations of their bioactivities*, Materials today, Communications 33 (2022) 104183.

[2] [2] M. Moradi Pour, R. Saberi Riseh, R. Ranjbar-Karimi, M. Hassanisaadi, A. Rahdar, F. Baino, *Microencapsulation of Bacillus velezensis using alginate-gum polymers enriched with TiO_2 and SiO_2 nanoparticles*, Micromachines 13 (9) (2022) 1423.

[3] [3] R. Saberi Riseh, M. Ebrahimi-Zarandi, M. Gholizadeh Vazvani, Y.A. Skorik, *Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides*, Int. J. Mol. Sci. 22 (23) (2021) 12979.

[4] [4] R. Saberi Riseh, M. Ebrahimi-Zarandi, E. Tamanadar, M. Moradi Pour, V. K. Thakur, *Salinity stress: toward sustainable plant strategies and using plant growth-promoting rhizobacteria encapsulation for reducing it*, Sustainability 13 (22) (2021) 12758.

[5] [5] R. Saberi Riseh, E. Tamanadar, M.M. Pour, V.K. Thakur, *Novel approaches for encapsulation of plant probiotic bacteria with sustainable polymer gums: application in the management of pests and diseases*, Adv. Polym. Technol. 2022 (2022) 1-10.

[6] [6]. R. Saberi-Rise, M. Moradi-Pour, *The effect of Bacillus subtilis Vru1 encapsulated in alginate-bentonite coating enriched with titanium nanoparticles against Rhizoctonia solani on bean*, Int. J. Biol. Macromol. 152 (2020) 1089-1097.

[7] [7] M. Ebrahimi-Zarandi, R. Saberi Riseh, M.T. Tarkka, *Actinobacteria as effective bio-control agents against plant pathogens, an overview on their role in eliciting plant defense*, Microorganisms 10 (9) (2022) 1739.

[8] [8] R. Saberi Riseh, H. Dashti, M. Gholizadeh Vazvani, A. Dini, *Changes in the activity of enzymes phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase in some wheat genotypes against take-all disease*, Journal of agricultural, Sci. Technol. 23 (4) (2021) 929-942.

[9] [9] R. Saberi Riseh, M. Gholizadeh Vazvani, N. Hajabdollahi, V.K. Thakur, *Bioremediation of heavy metals by Rhizobacteria*, Appl. Biochem. Biotechnol. 195 (8) (2023) 4689-4711.

[10] [10] R. Saberi-Riseh, H. Dashti, M. Gholizadeh-Vazvani, *Association between agronomic traits and molecular markers with take-all disease severity in bread wheat Triticum aestivum*, Journal of Crop Protection 11 (1) (2022) 39-59.

[11] [11] Riseh, R.S.; Tamanadar, E.; Hajabdollahi, N.; Vatankhah, M.; Thakur, V.K.; Skorik, Y.A., *Chitosan microencapsulation of rhizobacteria for biological control of plant pests and diseases: Recent advances and applications*, Rhizosphere (2022), 23, 100565.

[12] [12] M. Garcá-Carrasco; O. Valdez-Baro; Luis A. Cabanillas-Bojrquez; Manuel J. Bernal-Milln; Mara M. Rivera-Salas; Erick P. Gutirrez-Grijalva; and J. Basilio Heredia, *Potential Agricultural Uses of Micro/Nano Encapsulated Chitosan: A Review*, Macromol (2023), 3, 614-635.

[13] [13] Ding, X.; Zhao, L.; Khan, I.M.; Yue, L.; Zhang, Y.; Wang, Z., *Emerging chitosan grafted essential oil components: A review on synthesis, characterization, and potential application*, Carbohydr. Polym. (2022), 297, 120011

[14] [14] Koci?ecka, J.; Liberacki, D, *The Potential of Using Chitosan on Cereal Crops in the Face of Climate Change*, Plants (2021), 10, 1160.

[15] [15] Kolesnikov, L.E.; Popova, E.V.; Novikova, I.I.; Kolesnikova, Y.R.; Balagurova, E.D, *Application of Chitosan to Protect Wheat from Diseases and Boost Yields*, Appl. Biochem. Microbiol. (2022), 58, 329-335.

[16] [16] Maluin, F.N.; Hussein, M.Z, *Chitosan-Based Agronanochemicals as a Sustainable Alternative in Crop Protection*, Molecules (2020), 25, 1611.

[17] [17] Mondal, M.M.A.; Puteh, A.B.; Dafader, N.C.; Malek, M.I.A.; Ehsan, S.D, *Foliar application of Chitosan improves growth and yield in maize*, J. Food Agric. Environ. (2013), 11, 520-523.

[18] [18] Kong, M.; Chen, X.G.; Xing, K.; Park, H.J *Antimicrobial properties of Chitosan and mode of action: A state of the art review*, Int. J. Food Microbiol. (2010), 144, 51-63.

[19] [19] Yahya Faqir, Jiahua Ma, Yunlong Chai, *Chitosan in modern agriculture production*, Plant, Soil and Environment (2021), 12, 679-699.

[20] [20] L.M. Alnaddaf, S.F. Bamsaoud, M. Bahwirth, *Perspective Chapter: Application of Nanotechnology Solutions in Plants Fertilization and Environmental Remediation*, 2023.

[21] [21] V.K. Upadhayay, M.K. Chitara, D. Mishra, M.N. Jha, A. Jaiswal, G. Kumari, S. Ghosh, V.K. Patel, M.G. Naitam, A.K. Singh, *Synergistic impact of nanomaterials and plant probiotics in agriculture: a tale of two-way strategy for long-term sustainability*, Front. Microbiol. 14 (2023) 1133968.

[22] [22] A. Perez-de-Luque, *Interaction of nanomaterials with plants: what do we need for real applications in agriculture?* , Front. Environ. Sci. 5 (2017) 12.

[23] [23] R. Saberi Riseh, M. Hassanisaadi, M. Vatankhah, F. Soroush, R.S. Varma, *Nano/ micro- encapsulation of plant biocontrol agents by chitosan, alginate, and otherbimportant biopoly mers as a novel strategy for alleviating plant biotic stresses*, Int. J. Biol. Macromol. 222 (2022) 1589-1604.

[24] [24] Roohallah Saberi Riseh, Masoumeh Vatankhah, Mohadeseh Hassanisaadi, Rajender S. Varma, *A review of chitosan nanoparticles: Nature's gift for transforming agriculture through smart and effective delivery mechanisms*, International Journal of Biological Macromolecules 260 (2024) 129522.

[25] [25] Prasad R Deshmukh, Abhilash Joshi, Chaitanya Vikhar, SS Khdabadi, Mukund Tawar, *Current Applications of Chitosan Nanoparticles*, Sys Rev Pharm (2022); 13(10): 685-693.

[26] [26] Pramod U. Ingle, Sudhir S. Shende, Prashant R. Shingote, Suchitra S. Mishra, Vaidehi Sarda, Dhiraj L. Wasule , Vishnu D. Rajput, Tatiana Minkina, Mahendra Rai, Svetlana Sushkova, Saglara Mandzhieva, Aniket Gade, *Chitosan nanoparticles (ChNPs): A versatile growth promoter in modern agricultural production*, Heliyon 8 (2022) e11893.

[27] [27] Subhani Bandara, Hongbo Du, Laura Carson, Debra Bradford and Raghava Kommalapati, *Agricultural and Biomedical Applications of Chitosan-Based Nanomaterials*, Nanomaterials (2020), 10, 1903.

[28] [28] R. Roman-Doval, S.P. Torres-Arellanes, A.Y. Tenorio-Barajas, A. Gomez-Sanchez, A.A. Valencia-Lazcano, *Chitosan: properties and its application in agriculture in context of molecular weight*, Polymers 15 (13) (2023) 2867.

[29] [29] M. Muthu, J. Gopal, S. Chun, A.J.P. Devadoss, N. Hasan, I. Sivanesan, *Crustacean waste-derived chitosan: antioxidant properties and future perspective*, Antioxidants 10 (2) (2021) 228.

[30] [30] R. Vani, S.A. Stanley, *Studies on the extraction of chitin and chitosan from different aquatic organisms*, Adv. Biotech. 12 (12) (2013) 12-15.

[31] [31] Zargar V., Asghari M., Dashti A, *A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications*, ChemBioEng Reviews (2015), 2: 204-226.

[32] [32] Song, H.; Yuan, W.; Jin, P.; Wang, W.; Wang, X.; Yang, L.; Zhang, Y, *Effects of chitosan/nano-silica on postharvest quality and antioxidant capacity of loquat fruit during cold storage*, Postharvest Boil. Technol. (2016), 119, 41-48.

[33] [33] Negm, A.; Hefni, H.H.; Abd-Elaal, A.A.; Badr, E.A.; Kana, M.T.A, *Advancement on modification of chitosan biopolymer and its potential applications*, Int. J. Boil. Macromol. (2020), 152, 681-702.

[34] [34] M. Hassanisaadi, G.H.S. Bonjar, A. Rahdar, S. Pandey, A. Hosseinipour, R. Abdolshahi, *Environmentally safe biosynthesis of gold nanoparticles using plant water extracts*, Nano- materials (2021) 11 (8) 2033.

[35] [35] Buzea C, Pacheco II, Robbie K, *Nanomaterials and nanoparticles: Sources and toxicity*, Biointerphases (2007); 2(4): 17-71.

[36] [36] Pola CC, Moraes AR, Medeiros EA, Tefilo RF, Soares NF, Gomes CL, *Development and optimization of pH-responsive PLGA-chitosan nanoparticles for triggered release of antimicrobials*, Food Chem. (2019), 295: 671-679.

[37] [37] M.S. Muda, A. Kamari, S.A. Bakar, et al., *Chitosan-graphene oxide nanocomposites as water-solubilising agents for rotenone pesticide*, Journal of Molecular Liquids (2020), 318, 114066.

[38] [38] H. Wu, Z. Li, *Nano-enabled agriculture: how nanoparticles cross barriers in plants?*, plant, Communications 3 (2022) 100346.

[39] [39] S.M. Bashir, G. Ahmed Rather, A. Patrcio, Z. Haq, A.A. Sheikh, M.Z.U.H. Shah, H. Singh, A.A. Khan, S. Imtiyaz, S.B. Ahmad, *Chitosan nanoparticles: a versatile platform for biomedical applications*, Materials 15 (19) (2022) 6521.

[40] [40] Y. Ciro, J. Rojas, M.J. Alhajj, G.A. Carabali, C.H. Salamanca, *Production and characterization of chitosan polyanion nanoparticles by polyelectrolyte complexation assisted by high-intensity sonication for the modified release of methotrexate*, Pharmaceuticals 13 (1) (2020) 11.

[41] [41] E.M. Hejjaji, A.M. Smith, G.A. Morris, *Evaluation of the mucoadhesive properties of chitosan nanoparticles prepared using different chitosan to tripolyphosphate (CS: TPP) ratios*, Int. J. Biol. Macromol. 120 (2018) 1610-1617.

[42] [42] N. Van Bavel, T. Issler, L. Pang, M. Anikovskiy, E.J. Prenner, *A simple method for synthesis of chitosan nanoparticles with ionic gelation and homogenization*, Molecules 28 (11) (2023) 4328.

[43] [43] P. Sacco, S. Pedroso-Santana, Y. Kumar, N. Joly, P. Martin, P. Bocchetta, *Ionotropic gelation of chitosan flat structures and potential applications*, Molecules 26 (3) (2021) 660.

[44] [44] R. Saberi Riseh, M. Moradi Pour, E. Ait Barka, *A novel route for double-layered encapsulation of Streptomyces fulvissimus Uts22 by alginate Arabic gum for controlling of Pythium aphanidermatum in cucumber*, Agronomy 12 (3) (2022) 655.

[45] [45] R. Saberi Riseh, Y.A. Skorik, V.K. Thakur, M. Moradi Pour, E. Tamanadar, S. S. Noghabi, *Encapsulation of plant biocontrol bacteria with alginate as a main polymer material*, Int. J. Mol. Sci. 22 (20) (2021) 11165.

[46] [46] R. Saberi-Riseh, M. Moradi-Pour, *A novel encapsulation of Streptomyces fulvissimus Uts22 by spray drying and its biocontrol efficiency against Gaeumannomyces graminis, the causal agent of take-all disease in wheat*, Pest Manag. Sci. 77 (10) (2021) 4357-4364.

[47] [47] M. Yanat, K. Schroen, *Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging*, React. Funct. Polym. 161 (2021) 104849.

[48] [48] D. Santos, A.C. Maurcio, V. Sencadas, J.D. Santos, M.H. Fernandes, P.S. Gomes, *Spray Drying: An Overview*, 2018.

[49] [49] W.W. Mwangi, K.-W. Ho, C.-W. Ooi, B.-T. Tey, E.-S. Chan, *Facile method for forming ionically cross-linked chitosan microcapsules from Pickering emulsion templates*, Food Hydrocoll. 55 (2016) 26-33.

[50] [50] E.F. Ribeiro, T.T. de Barros-Alexandrino, O.B.G. Assis, A.C. Junior, A. Quiles, I. Hernando, V.R. Nicoletti, *Chitosan and crosslinked chitosan nanoparticles: synthesis, characterization and their role as Pickering emulsifiers*, Carbohydr. Polym. 250 (2020) 116878.

[51] [51] A. Napiorkowska, M. Kurek, *Coacervation as a novel method of microcapsulation of essential oils - a review*, Molecules 27 (16) (2022) 5142.

[52] [52] S.P. Moulik, A.K. Rakshit, A. Pan, B. Naskar, *An overview of coacervates: the special disperse state of amphiphilic and polymeric materials in solution*, Colloids Interfaces 6 (3) (2022) 45.

[53] [53] M.S. Orellano, G.S. Longo, C. Porporatto, N.M. Correa, R.D. Falcone, *Role of micellar interface in the synthesis of chitosan nanoparticles formulated by reverse micellar method*, Colloids Surf. A: Physicochem. Eng. 599 (2020) 124876.

[54] [54] Y. Wang, P. Li, T. Truong-Dinh Tran, J. Zhang, L. Kong, *Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer*, Nanomaterials 6 (2) (2016) 26.

[55] [55] F. Sami El-banna, M.E. Mahfouz, S. Leporatti, M. El-Kemary, N.A.N. Hanafy, *Chitosan as a natural copolymer with unique properties for the development of hydrogels*, Appl. Sci. 9 (11) (2019) 1-11.

[56] [56] A.F. Sahab, A.I. Waly, M.M. Sabbour, L.S. Nawar, *Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles against some seed borne fungi and insects of soybean*, Int. J. ChemTech Res. (2015) 8 (2) 589-598.

[57] [57] F.N. Maluin, M.Z. Hussein, *Chitosan-based agronanochemicals as a sustainable alternative in crop protection*, Molecules (2020) 25 (7) 1611.

[58] [58] Chaudhari, A.K.; Singh, V.K.; Das, S.; Deepika; Prasad, J.; Dwivedy, A.K.; Dubey, N.K, *Improvement of in vitro and in situ antifungal, AFB1 inhibitory and antioxidant activity of Origanum majorana L. essential oil through nanoemulsion and recommending as novel food preservative*, Food Chem. Toxicol. (2020), 143, 111536.

[59] [59] Mondjar-Lpez, M.; Rubio-Moraga, A.; Lpez-Jimenez, A.J.; Garca Martnez, J.C.; Ahrazem, O.; Gmez-Gmez, L.; Niza, E, *Chitosan nanoparticles loaded with garlic essential oil: A new alternative to tebuconazole as seed dressing agent*, Carbohydr. Polym. (2022), 277, 118815.

[60] [60] Mohammadi, A.; Hashemi, M.; Hosseini, S.M, *Nanoencapsulation of Zataria multiflora essential oil preparation and characterization with enhanced antifungal activity for controlling Botrytis cinerea, the causal agent of gray mould disease*, Innov. Food Sci. Emerg. Technol. (2015), 28, 73-80.

[61] [61] Hesami, G.; Darvishi, S.; Zarei, M.; Hadidi, M, *Fabrication of chitosan nanoparticles incorporated with Pistacia atlantica subsp. kurdica 'hulls' essential oil as a potential antifungal preservative against strawberry grey mould*, Int. J. Food Sci. Technol. (2021), 56, 4215-4223.

[62] [62] Hadidi, M.; Motamedzadegan, A.; Jelyani, A.Z.; Khashadeh, S, *Nanoencapsulation of hyssop essential oil in chitosan-pea protein isolate nano-complex*, LWT (2021), 144, 111254.

[63] [63] Payandeh, M.; Ahmadyousefi, M.; Alizadeh, H.; Zahedifar, M,*Chitosan nanocomposite incorporated Satureja kermanica essential oil and extract: Synthesis, characterization and antifungal assay*, Int. J. Biol. Macromol. (2022), 221, 1356-1364.

[64] [64] Yilmaz, M.T.; Yilmaz, A.; Akman, P.K.; Bozkurt, F.; Dertli, E.; Basahel, A.; Al-Sasi, B.; Taylan, O.; Sagdic, O, *Electrospraying method for fabrication of essential oil loaded-chitosan nanoparticle delivery systems characterized by molecular, thermal, morphological and antifungal properties*, Innov. Food Sci. Emerg. Technol. (2019), 52, 166-178.

[65] [65] Amighi, M.; Zahedifar, M.; Alizadeh, H.; Payandeh, M, *Encapsulation of Nepeta hormozganica and Nepeta dschuprensis essential oils in shrimp chitosan NPs: Enhanced antifungal activity*, Int. J. Biol. Macromol. (2023), 238, 124112.

[66] [66] Izadi, M.; Moosawi Jorf, S.A.; Nikkhah, M.; Moradi, S,*Antifungal activity of hydrocolloid nano encapsulated Carum copticum essential oil and Peganum harmala extract on the pathogenic fungi Alternaria alternate*, Physiol. Mol. Plant Pathol. (2021), 116, 101714.

[67] [67] Karimirad R, Behnamian M, Dezhsetan S, *Application of chitosan nanoparticles containing Cuminum cyminum oil as a delivery system for shelf life extension of Agaricus bisporus*, Lwt. (2019); 106: 218-228.

[68] [68] Abdallah, Y.; Liu, M.; Ogunyemi, S.O.; Ahmed, T.; Fouad, H.; Abdelazez, A.; Yan, C.; Yang, Y.; Chen, J.; Li, B,*Bioinspired Green Synthesis of Chitosan and Zinc Oxide Nanoparticles with Strong Antibacterial Activity against Rice Pathogen Xanthomonas oryzae pv. oryzae,* Molecules (2020), 25, 4795.

[69] [69] Badawy, M.E.I.; Rabea, E.I.; Eid, A.R.; Badr, M.M.; Marei, G.I.K,*Structure and antimicrobial comparison between N-(benzyl) chitosan derivatives and N-(benzyl) chitosan tripolyphosphate nanoparticles against bacteria, fungi, and yeast*, Int. J. Biol. Macromol. (2021), 186, 724-734.

[70] [70] Du, M.; Yi, Y.; Yin, Y.; Cai, Z.; Cai, W.; Li, J.; He, G.; Zhang, J, *Bacteria-triggered photodynamic nanosystem based on hematoporphyrin-modified Chitosan for sustainable plant disease control*, Eur. Polym. J. (2023), 191, 112035.

[71] [71] Mazzotta, E.; Muzzalupo, R.; Chiappetta, A.; Muzzalupo, I, *Control of the Verticillium Wilt on Tomato Plants by Means of Olive Leaf Extracts Loaded on Chitosan Nanoparticles*, Microorganisms (2022), 10, 136.

[72] [72] Soltanzadeh, M.; Peighambardoust, S.H.; Ghanbarzadeh, B.; Mohammadi, M.; Lorenzo, J.M, *Chitosan Nanoparticles as a Promising Nanomaterial for Encapsulation of Pomegranate (Punica granatum L.) Peel Extract as a Natural Source of Antioxidants*, Nanomaterials (2021), 11, 1439.

[73] [73] Zou, L.-Q.; Liu,W.; Liu,W.-L.; Liang, R.-H.; Li, T.; Liu, C.-M.; Cao, Y.-L.; Niu, J.; Liu, Z, *Characterization and Bioavailability of Tea Polyphenol Nanoliposome Prepared by Combining an Ethanol Injection Method with Dynamic High-Pressure Microfluidization*, J. Agric. Food Chem. (2014), 62, 934-941.

[74] [74] Neil Ramsden, *Vietnamese firm sees gold in 1,000t-per-day of shrimp 'waste'*, Undercurrent News (2022).

[75] [75] Muñoz, I.; Rodrguez, C.; Gillet, D.; Moerschbacher, *B.M, Life cycle assessment of chitosan production in India and Europe*, Int. J. Life Cycle Assess (2018), 23, 1151-1160