__Review Article__

CHITOSAN NANOPARTICLES AS BIOCOMPATIBLE TOOL IN AGRICULTURE

ABSTRACT

Chitosan nanoparticles (CNPs) are gaining attention in agriculture due to their sustainable properties and multifunctional applications. Derived from chitin, a naturally abundant polymer found in the shells of crustaceans, these nanoparticles, exhibit biocompatibility, biodegradability, and antimicrobial activity, making them suitable for enhancing agricultural productivity while minimizing environmental impact. When reduced to nanoparticle size, chitosan demonstrates enhanced bioactivity, making it an effective tool in sustainable agricultural practices. CNPs are utilized in various applications such as plant growth promotion, pest control, disease management, and soil health improvement.

Key words: Chitosan nanoparticles (CNPs), chitin, sustainable agriculture, biocompatibility.

1. INTRODUCTION

Development of nontoxic and bio-based nanomaterials exhibiting better compatibility toward plant systems leads to the development of nano-formulations of different biogenic materials like chitin, chitosan, cellulose, starch, gelatin, etc. for agricultural applications. Modern seafood processing practices result in production of a large volume of waste products which contains a huge amount of chitin, a polysaccharide which can form chitosan. Among an extensive array of biopolymers, the involvement of chitosan in agricultural applications is gaining momentum due to its biodegradability, biocompatibility, nontoxicity, and cost effectiveness. Chitosan is gaining popularity because it satisfies environmental criteria, such as being an eco-friendly chemical that facilitates the effective use of reagents while minimizing possible waste

2. CHITIN AND CHITOSAN

It has been estimated that at least 1.1 x 10^{13} kg of chitin is present in the biosphere. However, its use has been limited because it is insoluble in most solvents and relatively difficult to isolate from natural sources in pure form under economically viable conditions. Chitosan is a natural-based biopolymer derived from chitin. Cellulose, chitin, and chitosan share very similar backbone structures. The difference among these three molecules is the functional group at the C-2 position. A molecular chain of chitin consists of linear structures of 2-acetamido-2-deoxy-β-D-glucose through β (1→4) linkage, by replacing hydroxyl group at the C-2 position in cellulose molecular chain with acetamido group. Chitosan is the chitin derivative produced by the N-deacetylation process, resulting in the amino group at the C-2 position on its backbone.

Among an extensive array of biopolymers, the involvement of chitosan in agricultural applications is gaining momentum owing to its biodegradability, biocompatibility, nontoxicity, plant growth regulation, antimicrobial activity, and stress inhibitory activity in plants. Alongside, it is also regarded as an economical biopolymer as it can be effortlessly retrieved from marine wastes, insects, and fungi.

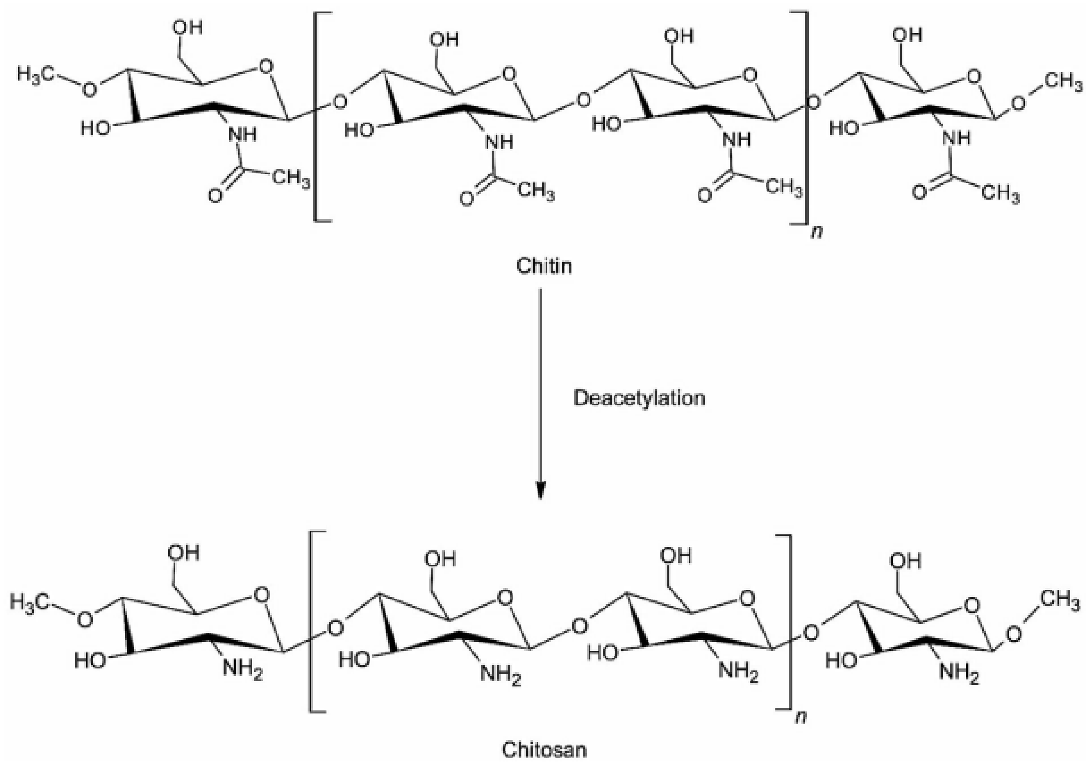


Fig.1: Chemical structure of chitin and chitosan

3. HISTORY

Chitin, originally called fungine, was first isolated from fungi by French researcher Henri Braconnot. 20 years later, the same compound was isolated from insect cuticle by Antoine Odier and named chitin. In turn, chitosan was discovered accidentally in 1859 by French physiologist Rouget during the production of natural soap. The modified form of chitin was eventually named “chitosan” by German chemist and physiologist Felix Hoppe-Seyler in 1894 and the chemical structure of this compound was determined only in the mid-20th century (Stasinska-Jakubas et al., 2022).

Chitosan can be extracted from insects, yeast, mushroom, cell wall of fungi, and marine shellfish such as crab, lobster, krill, cuttlefish, shrimp, and squid pens. In shellfish, chitin forms the outer protective coating as a covalently bound network with proteins and some metals and carotenoids. Shrimps are in general sold headless and often peeled of the outer shells and tail. Crustaceansshells consist of 30–40 % proteins, 30–50 % calcium carbonate, and 20–30 % chitin and also contain pigments (astaxanthin, canthaxanthin, lutein, and b-carotene). These proportions vary with species and with seasons. Shrimp, prawn, and crab wastes are the principal source of commercial chitin and chitosan production. The increase in consumption of shellfish and the expansion of aquaculture have led to a tremendous increase in the quantity of shrimp and prawn being processed and hence in the amount of waste available for chitin/chitosan production.

Agriculture is one promising area of application for chitosan-based nanoparticles as a controlled-release system. Agriculture and the food it produces are becoming increasingly important in a world of diminishing resources and an ever-increasing global population. So it is necessary to use modern technologies such as nanotechnology and nanobiotechnology in agriculture and food sciences (Sekhon 2014)

4. EXTRACTION OF CHITOSAN FROM CHITIN

Chitosan can be extracted from insects, yeast, mushroom, cell wall of fungi, and marine shellfish such as crab, lobster, krill, cuttlefish, shrimp, and squid pens. Production of chitosan from crustacean shell generally consists of four basic steps viz., demineralization, deproteinization, decolorization, and deacetylation (Yadav et al., 2019). Even though chemical extraction is eco-unfriendly and uneconomical process which adversely affects the physical and chemical properties of chitin, and removes minerals and proteins, still, it is the most commonly applied method on commercial scale. However, due to these disadvantages, biological extraction has been recently attracting interest, since it is cheaper and safer treatment for chitin retrieval but limited to laboratory scale only (Dhillon et al., 2013). The exoskeleton of crustacean is a major starting material used for commercial production of chitosan.

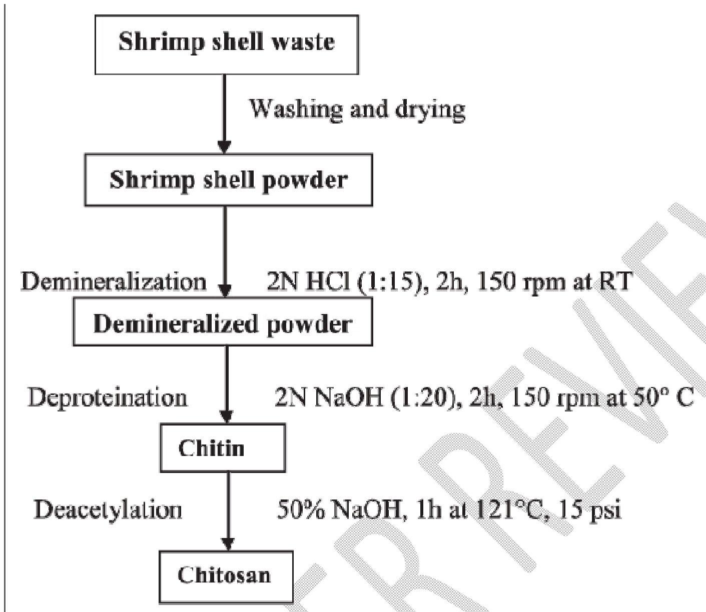


Fig.2: Extraction and preparation of chitosan and chitosan derivatives

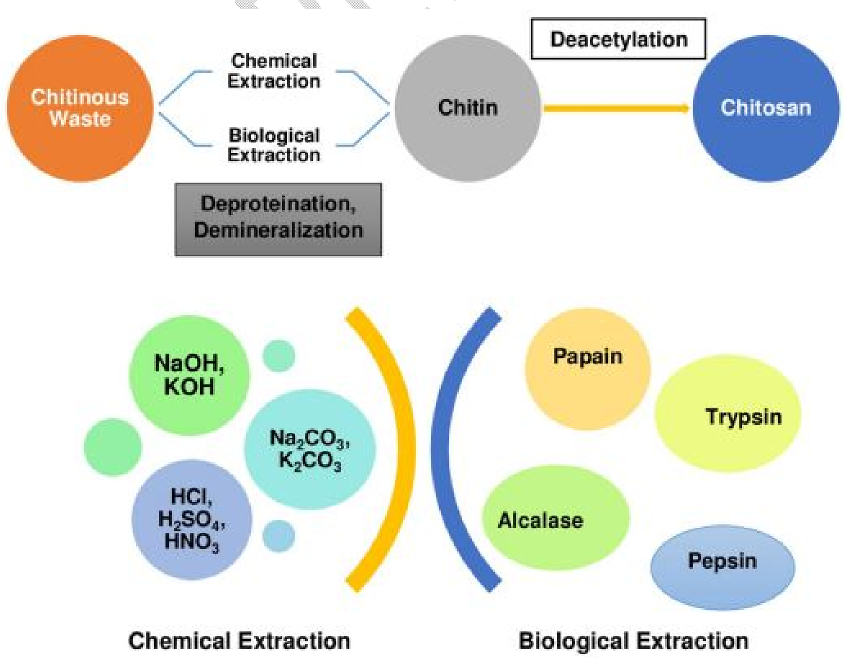


Fig.3: Chemical and biological extraction of chitosan

4.1 CHEMICAL EXTRACTION

Shells obtained from diverse sources are subjected to washing and drying followed by crushing into powder. The chemical extraction methods include three steps: Deproteination, demineralization and decolorization.

4.1.1 DEMINERALIZATION

Demineralization typically uses acids like hydrochloric acid, nitric acid, acetic acid, or formic acid (up to 10%) at room temperature and agitation to dissolve calcium carbonate into calcium chloride. Using diluted hydrochloric acid (1%–8%) at room temperature, demineralization for 1–3 h yields significant amounts of calcium chloride. Most of the time, a 1:15 (w/v) solid-to-solvent ratio is used. The effectiveness of the demineralization process can be gauged by the amount of ash in the demineralized shell.

4.1.2 DEPROTEINIZATION

Chitin happens normally in relationship with protein. Covalent bonds between the protein and chitin, result in the formation of stable complexes (Attwood and Zola, 1967). The protein separates from the solid portion of the shrimp waste and is treated with sodium or potassium hydroxide at 65–100^{o}C with a minimum shell-to-alkali ratio of 1:4 for periods ranging from one to twelve hours. By filtering the solids from the protein slurry after the deproteinization step is finished, the protein hydrolysate can be easily removed.

4.1.3 DECOLORIZATION

Chitin obtained after the demineralization and deproteinization of shell waste is a colored product. For commercial acceptability, the chitin should be decolorized or dyed to yield cream whitechitin powder. The degree to which chitin and pigments are linked varies among crustacean species. Solvents and/or oxidants are used to remove colour from the residues. During the course of decoloration, the compound utilized shouldn't influence thephysicochemical of chitin and chitosan.

4.1.4 DEACETYLATION

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. There are several critical factors that affect the extent of deacetylation including temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, atmosphere (air or nitrogen), ratio of chitin to alkali solution, density of chitin, and the particle size. Considering all these as necessary conditions, the ideal process condition of deacetylation should yield a chitosan that is not degraded and is soluble in dilute acetic acid in minimal time (Muzzarelli *et al.,* 1980).

The N-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for N-deacetylation. Severe alkaline hydrolysis treatments are required due to the resistance of groups imposed by the trans arrangement of the C2-C3 substituents in the sugar ring. It is generally achieved by treatment with concentrated sodium or potassium hydroxide solution (40–60 %) usually at 80–140 ^{o}C for 30 min or longer using a solid-to-solvent ratio of 1:10 (w/v) to remove some or all of the acetyl groups from the polymer. Sodium hydroxide is the preferred alkali. After deacetylation, the chitosan is washed to completely remove alkali and is dried to give flakes. The material should be low in protein and ash. Production of chitosan by chemical processes has several disadvantages such as environmental pollution, inconsistent molecular weights, and degree of acetylation.

4.2 BIOLOGICAL EXTRACTION

Chitosan NPs can be synthesized by biological method with the help of different biomolecules. Therefore, use of biological process for chitin extraction is gaining immense attention as it is cleaner, eco-friendly and economic along with production of chitin and chitosan with desired properties. Khanafari et al. (2008) reported a comparative study on shrimp shells for removal of chitin by biological and chemical processes. The outcomes indicated that the biological extraction process was superior to the chemical method, since it not only preserved the chitin structure but also was eco-friendly. Chitosan nanoparticles (ChNPs) with high antifungal activities are obtained through biological processes.A number of microorganisms such as *Bacillussubtilis,Lactobacillus helveticus*, *Pseudomonas aeruginosa*, *Lactobacillus paracasei*, *Lecanicilliumfungicola*, and *Penicillium chrysogenum* have been utilized for demineralization (Choorit et al., 2008)

Table. 1 Physiochemical properties of chitin and chitosan

Properties	Chitin	Chitosan
Structure	Poly-(2-acetamido-deoxy-D-glucose)_	Poly-(2-amino-deoxy-D-glucose)_
Deacetylation degree	Less than 50	80-95
Molecular weight	100-1000	20-750
Solubility	Less soluble	More soluble
Reactive groups	Hydroxyl and carboxyl groups	Amino and hydroxyl groups

5. CHITOSAN NANOPARTICLES

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range 1–1,000 nm (Zhao et al*.,* 2011).

5.1 PROPERTIES OF CHITOSAN NANOPARTICLES

- Materials at the nanoscale (10 to 1000 nm) (Hassanisaadi et al.,2021)
- More efficient with lesser molecular weights
- High solubility
- Improved bioavailability
- Greater surface area to volume ratio
- More penetration into the plant system

Simply, the nanoparticles prepared using chitosan or its derivatives can be called chitosan nanoparticles. Being a linear polyamine containing a number of free amine groups that are readily available for cross-linking and the cationic nature allowing for ionic cross-linking with multivalent anions (Agnihotri et al., 2004) can be considered as the key factors which make chitosan significant in producing nanoparticles. Chitosan nanoparticles exhibit regular assemblage shapes such as snowflakes, round shape, and spherical shape. Several studies have confirmed that compounds at the nanoscale are more effective than bulk materials (Hassanisaadi et al., 2023; Jha and Mavanovic, 2023)

5.2 SYNTHESIS OF CHITOSAN NANO PARTICLES

Bottom-up approach: synthesis of nanoparticles by means of chemical reactions among the atoms/ions/molecules

Top-down approach: involves the mechanical methods to crush/breaking of bulk into several parts to form nanoparticles

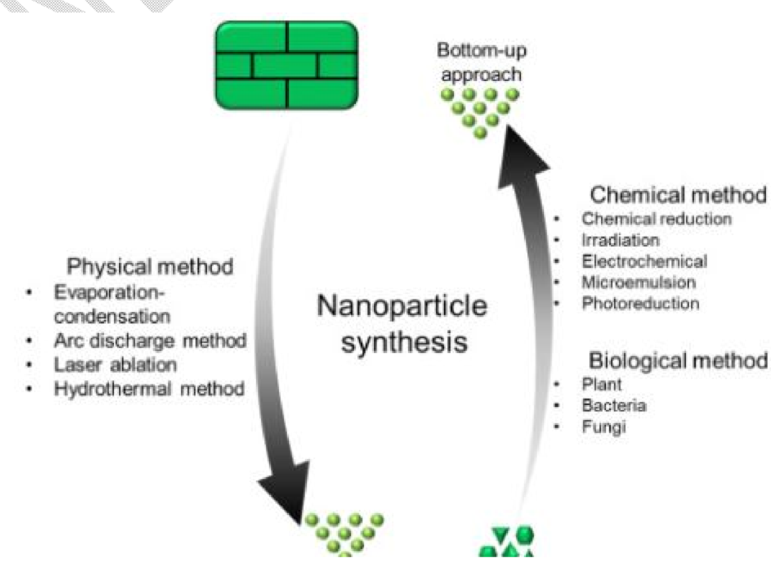


Fig.4: Top down and bottom up approach in nanoparticle synthesis

5.2.1 IONIC GELATION METHOD

Chitosan nanoparticles have been investigated for more than two decades and ionic gelation is one of the most preferred preparation methods, which has first been described by Calvo et al., (1997).

Ionic gelation involves dissolving chitosan, which is positively charged, in an acetic acid solution at room temperature and magnetically stirring for an hour. A second solution is made using polyanion tripolyphosphate (TPP) dissolved in deionized water (Bashir *et al.,* 2022). Generally, TPP is used as an ionic cross-linker. The ionic gelation synthesis involves mixing an aqueous solution containing chitosan and another containing TPP, thus resulting in a complex coacervate aqueous phase. The TPP–chitosan mixture needs to be magnetically stirred at room temperature. The solution results in three individual phases depending upon the stage of the procedure, starting with clear (chitosan solution), followed by opalescent or milky (after adding TPP to the chitosan solution), and, finally, aggregated (after adding more TPP to a milky solution), whereby the milky appearance is the sign of the formation of CNPs (Jha and Mavanovic 2023). Lastly, the resulting CNPs are usually cleaned to get rid of any leftover polyanion or unreacted chitosan. To create a dry powder that may be kept for later use, they are often freeze-dried or lyophilised (Hejjaji et al., 2018)

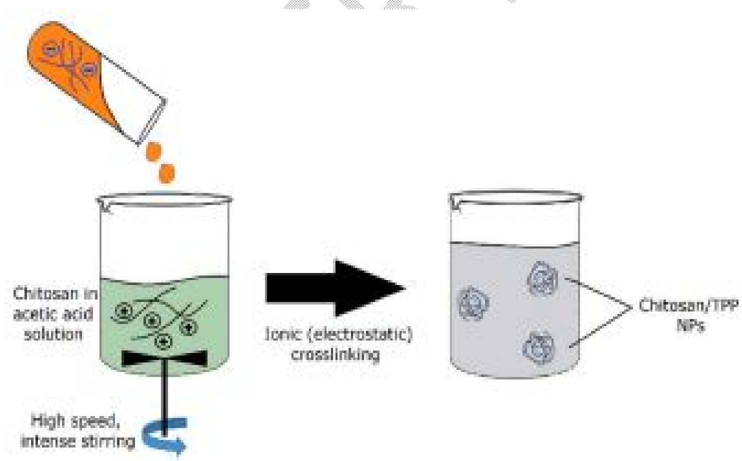


Fig.5: Ionic gelation method

6. APPLICATIONS OF CHITOSAN NANOPARTICLES IN AGRICULTURE

6.1) Delivery system for agrochemicals

6.2) Resistance against abiotic stress

6.3) Antimicrobial activity

6.4) Heavy metal detection and wastewater treatment

6.5) Post harvest management

NANOCARRIER

Nanocarrier is nanomaterial being used as a transport module for another substance- fertilizer, herbicide, pesticide etc. Chitosan nanoparticle is one among them.

BENEFITS OF NANOCARRIER

- Enhances the stability of active ingredients
- Agrochemicals can be applied at lower doses
- Reduces risk of environmental contamination
- Reduces toxic effects to other non-targeted organisms

6.1 DELIVERY SYSTEM FOR AGROCHEMICALS

Chitosan nanoparticles are being used for effective delivery of agrochemicals such as fertilizers, herbicides, pesticides *etc.* for the controlled release of active ingredients (Choudhary et al., 2019). It is possible to load several agricultural additives, such as fertilisers, insecticides, growth regulators, and micronutrients, onto the resultant microscopic particles with a wide surface area and high loading capacity by converting chitosan into nanoparticles.

The growth of plants largely depends upon the available water and nutrients. The betterment in fertilizer application is necessary for persistency of resources and viability of economy along with the sustainability of environment and agricultural ecosystem (Kashyap et al., 2015). The limitation of essential nutrients like NPK is a major concern since 40%- 90% of these nutrients are lost in the environment due to runoff, erosion, leaching, and immobilization when applied in bulk formulations. Combined application of chitosan nanoparticles with mineral nitrogen enhanced the productivity of wheat plants (Saad et al*.,* 2022).Elshamy et al. (2019) suggested the foliar application of chitosan nanoparticles loaded with nitrogen, phosphorus and potassium to increase the growth and yield of potato.For crops and the environment, these nanoparticles provide long-term benefits as an effective carrier system.
They provide progressive and sustained nutrient availability by protecting nutrients from deterioration and soil leakage through their exceptional biocompatibility and biodegradability. Chitosan is a great material for encapsulating macronutrients (N, P, K) and micronutrients (Cu, Zn, Fe, Si) because of its exceptional affinity for metals. Agricultural crops can perform better overall by maximising nutrient utilisation, increasing crop productivity, and efficiently utilising this special quality (Sharma et al*.,* 2022)

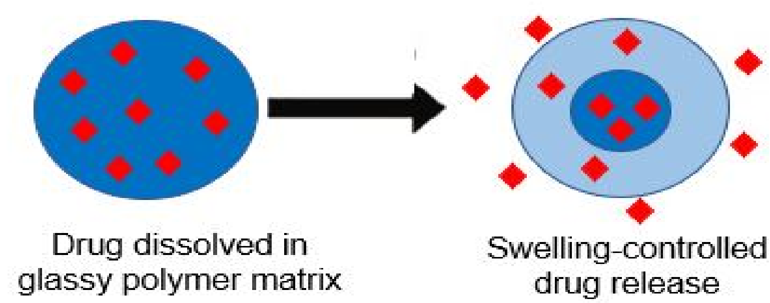


Fig.6: Release kinetics of agrochemical

Release kinetics

In agricultural systems - diffusion release and/or degradation release is the mechanism behind release of active ingredient.

(i) penetration of water into particulate system, which causes swelling of the matrix

(ii) conversion of a glassy polymer into a plasticized or rubbery swollen matrix

(iii) diffusion of compound from the swollen matrix

6.1.1 PESTICIDE DELIVERY FOR CROP PROTECTION

Rosemary (*Rosmarinus officinalis*) is a botanical species known by itsinsecticidal potential against insects during storage. Due to its properties such as volatility, absorption, bioavailability and tolerance to storage conditions such as temperature and oxygen, essential oils of rosemary are used as analternative way of pest control.Result showed that Cs NP loaded with rosemary essential oils showed higher toxicity with higher mortality percentage (Soltani et al., 2022)

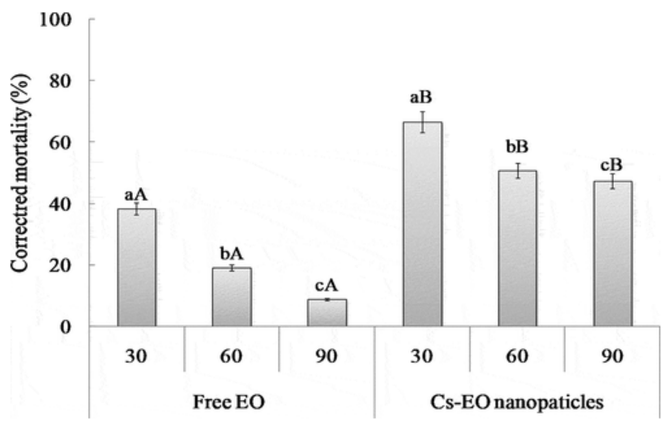


Fig.7: Effect of ChNP encapsulated rosemary essential oil against storage pest

6.2 RESISTANCE AGAINST ABIOTIC STRESS

Entry of nanoparticles into the cell happens either by penetration or by transportation through particular channels located in the cellular membrane. NPs might function as stress signaling molecules which, in turn, cause induction in the expression of various genes involved in stressed condition (Tortella et al*.,* 2023; Nawaz et al., 2023). This includes the induction of expression of regulatory factors thus resulting in activation of defense system, and finally, exhibiting stress tolerance. Besides an acceptable level, NPs can maintain ROS at considerable level to induce ROS signaling network hence activating defense system of plant under stress conditions. After exposure to NPs, the root architecture modification could be due to the downregulation of genes involved in trichoblast differentiation. This is the area from where the emergence of root hairs occurs hence trichoblasts come under specialized epidermal cells. Further, genes responsive to indole acetic acid (IAA) and ethylene (ET) were shown as the positive regulators of development of root hairs. NPs' treatment frequently alters biological pathways involved in defense mechanisms. NPs' treatment also upregulates genes that encode for proteins which play a primary role in ROS balance like NADPH oxidase, GST, superoxide dismutase (SOD), and peroxidases (POX) (Sarraf et al., 2022).

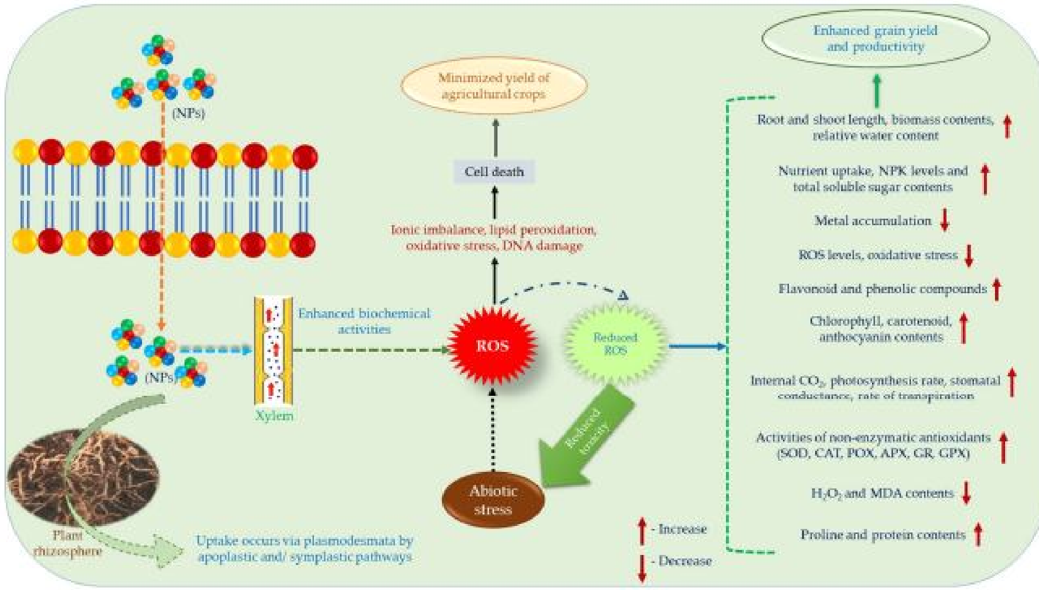


Fig.8: Schematic representation of uptake and impact of NPs during abiotic stress

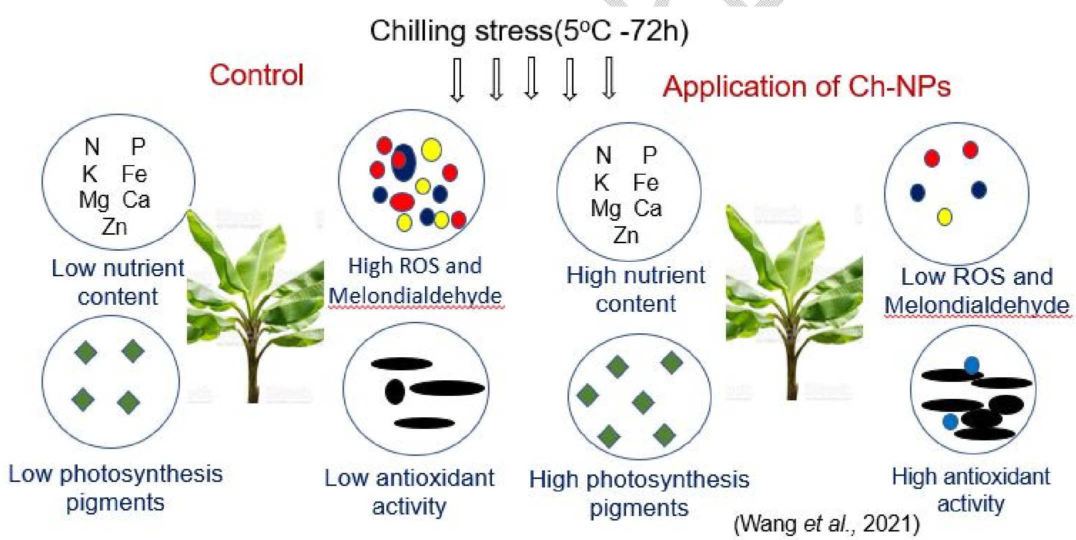


Fig.9: Chitosan nanoparticles against cold stress

Exposure of banana plants to low temperatures causes a severe drop in productivity, as they are sensitive to cold and do not have a strong defense system against chilling. Therefore, this study aimed to improve the growth and resistance to cold stress of banana plants using foliar treatments of chitosan nanoparticles. banana plants were sprayed with four concentrations of CNPsi.e., 0, 100, 200, and 400 mg L^{-1} of deionized water and a group that had not been cold stressed or undergone CNPs treatment was used as control. Banana plants were grown in a growth chamber and exposed to cold stress (5 °C for 72 h). A positive correlation was found between the foliar application of CNPs, on the one hand, and photosynthesis pigments and antioxidant enzyme activities on the other. Spraying banana plants with CNPs decreased malondialdehyde (MDA) and reactive oxygen species (ROS), i.e., hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and superoxide anions (O_2^-). CNPs (400 mgL^{-1}) decreased MDA, H_2O_2, OH, and O_2^- by 33, 33, 40, and 48%, respectively, compared to the unsprayed plants.

6.3 ANTIMICROBIAL ACTIVITY

The mechanism of antibacterial activity of CNPs is by the communications with either the bacterial cell wall or the cell membrane. In order to elucidate this mechanism, different hypothesis have been proposed. The most widely recognized CNPs model of antimicrobial action is the electrostatic communication between the amino groups of glucosamine (positively charged) with the cell membranes (negatively charged) of bacteria (Tsai and Su 1999). This interaction initiates prevalent variations to the surface of the cell, leading to a modification in membrane permeability which sequentially incites osmotic imbalance and efflux of intracellular substances that result in cell death. CNPs is capable of modifying the electron transport chain of bacteria. It binds with DNA and ruins DNA replication, leading to bacterial cell death. (Chandrasekaran et al*.,* 2020)

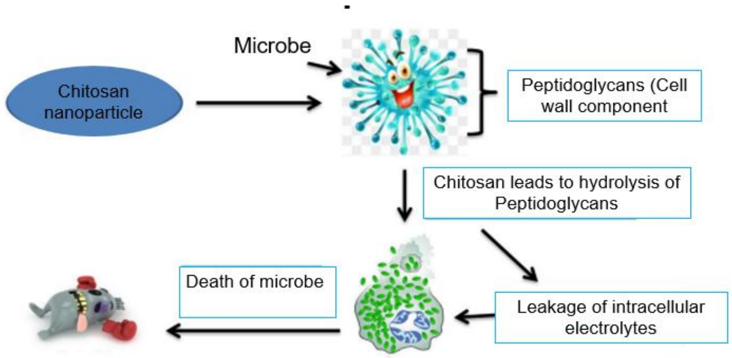


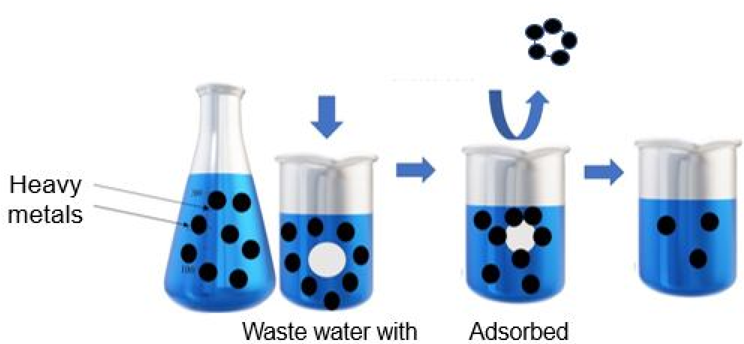
Fig.10: Antimicrobial action of Ch-NPs

In addition to their antibacterial qualities, CNPs can boost the synthesis of certain enzymes and secondary metabolites that strengthen a plant's defences against infections. Plant defences that are activated are better able to endure biotic stresses. (Zahrani et al., 2021). Alfy et al.2020 elucidated the nematicidal and insecticidal efficacy of carbon nanoparticle (CNP) applications against *Meloidogyne incognita*, *Spodoptera littoralis,* and *Locusta migratoria.*

Another investigation carried out by El Gamal et al., (2022) elucidated the capacity of CNPs to affect the structural integrity of viral particles, inhibit their replication and accumulation within plant tissues, andenhance the synthesis of defense-associated compounds in response to Bean yellow mosaic virus in faba beans.

6.4 HEAVY METAL DETECTION AND WASTEWATER TREATMENT

Heavy metals removal from wastewater is the most important because they not only contaminate the water bodies but are also toxic to the ecosystem as the majority of the heavy metals are non-degradable and highly toxic in nature (Das et al., 2018).Therefore their concentration has to be reduced to acceptable levels before discharging into the environment, or else these can pose a threat to human as well as animal health. Adsorption is a method that is preferred over other options because it is rapid, convenient, and inscrutable to toxic contaminants. It also has low initial costs, producing nontoxic byproducts, and rather simple in terms of design and operation of the treatment unit. Chitosan nanoparticles can be used as biosorbent in the heavy metal-containing wastewaters (Gamage et al., 2023). These nanoparticles additionally exhibit potential in the field of soil remediation, wherein they can enhance the extraction of pollutants from contaminated soil matrices (Saleh et al., 2022; Upadhyay et al*.,* 2021).



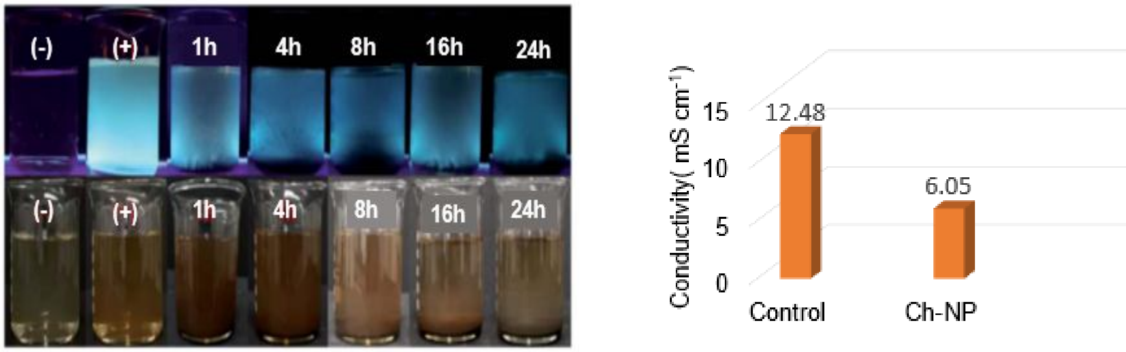


Fig.11: Effect of chitosan nanoparticle on wastewater treatment

Wang *et al*. (2021) prepared blue-green fluorescent carbon nanoparticles (chi-CNPs) with chitosan as the raw material, which realized the efficient removal of heavy metal ions. In addition, the removal effect of heavy metal ions can be evaluated by the fluorescence changes of chi-CNPs. The conductivity of the solution was measured by conductivity meter, and the conductivity decreased to 45.4% after adding chi-CNPs, which indicates that chi-CNPs could remove heavy metal ions in wastewater. After adding 0.01 g of chi-CNPs into wastewater, due to the chelation of heavy metal ions by chi-CNPs and their cross-linking polymerization, the total dissolved solids (TDS) accumulated at the bottom in the wastewater and the conductivity decreased to 6.05 mS cm−1. Under the irradiation of ultraviolet light, the wastewater added with chi-CNPs emits bright blue-green fluorescence and allowed to stand for 24 hours and there was chelate precipitation at the bottom. From 1 hour to 24 hours, the precipitation of heavy metal ions gradually increased, forming granular solids at first, and then slowly precipitating at the bottom. As precipitation formation, the fluorescence intensity changes from bright to dark under the ultraviolet excitation light with the wavelength of 365 nm. These results further confirmed the reliability of chi-CNPs in the removal of heavy metal ions in aqueous solutions.

Chitosan includes functional amino and hydroxyl groups, which makes these NPs interesting for the removal of a range of pollutants such as heavy metals, pesticides, and dyes. Besides, NPs may exhibit higher capacity than conventionally used micro-sized sorbents due to their higher surface area.

Chitosan nanoparticles have been utilized in filtration systems, which helps in the removal of heavy metal contaminants from wastewater streams, and this leads to the purification and remediation of polluted water bodies (Zhang et al. 2021).

6.5 Post harvest management

Chitosan nanoparticlesprotect the active ingredients from the environment for a specific period and reduces sweating, control weight loss, delay ripening, and increase vase life. A factorial experiment was carried out as a randomized complete design in three replications to investigate the efficiency of CNPs in quality improvement and longevity extension of cut rose flowers.

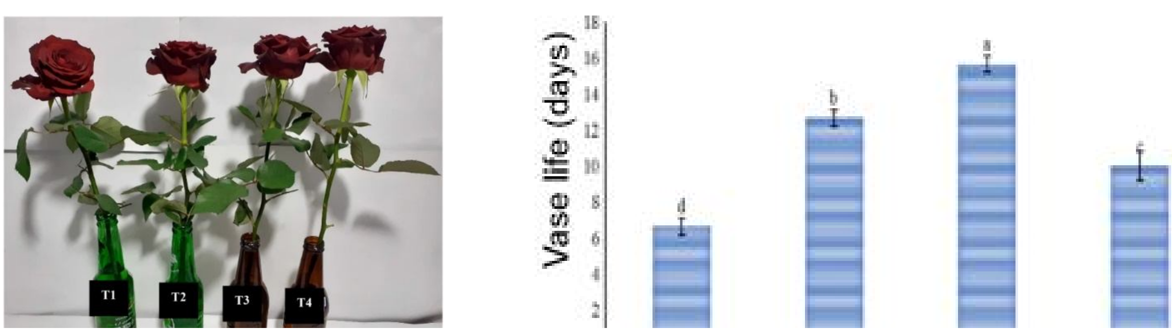


Fig.12: Effect of CNPs on vase life of cut flower

The 15-day maximum vase life was attained by the rose placed in a preservative solution containing 10 mg L^{-1} CNPs. CNPs also reduced microbial growth as compared to controls. Total phenolics, total flavonoids, and amount of anthocyanin in treated petals were dramatically increased. CNPs solutions especially at 10 and 15 mg L^{-1} concentrations, markedly reduced the H_2O_2 and malondialdehyde at the end of 15th day and maintained the membrane index. The protein and carbohydrate and petals anthocyanin content and enzymatic activities such as superoxide dismutase, polyphenol oxidase, peroxidase, catalase and ascorbate peroxidase increased in cut roses placed in 10 mg L^{-1} CNPs vase solution which in turn caused to increase in vase life(Hajizadeh et al., 2023).

Synthetic preservatives have long been used to preserve perishable foods, which has raised questions about their safety and potential health effects. Chitosan nanoparticles provide a safe and natural substitute because of their antimicrobial qualities and biocompatibility.(Cacciatoreet al., 2021; Atta et al., 2022) Without sacrificing flavor or quality, they guarantee food safety by preventing the growth of foodborne pathogens. Furthermore, the shelf life of a variety of food products is increased by their capacity to contain and release bioactive substances, like antioxidants, in a regulated manner. This dual purpose satisfies consumer preferences for clean-label and natural ingredients (Bangar et al., 2021).

Bananas are climacteric fruit and have a short shelf-life after-ripening process begin. Therefore, proper post-harvest handling is required to reduce the potential for physical damage and also extends the shelf-life of bananas. A popular method to prevent fast ripening of the fruits is using edible coating.

The edible coating is a thin layer made of components that are safe for consumption, which applied to the fruit surface as an addition or substitute for natural waxy coating. Edible films and coatings are applied on fruit to control moisture transfer, gas exchange (as a barrier to CO_2 and O_2). One of the best material for edible coating is chitosan. Chitosan could quickly form a layer on the fruit surface and reduces the respiration rate of the fruit by controlling carbon dioxide and oxygen permeability. In this study, we synthesised and characterised chitosan nanoparticle, as well as observed chitosan nanoparticle coating effect on fruit ripening process(Esyanti et al., 2019).

Hosain and Iqbal (2016) claim that because chitosan creates a semipermeable film, it may alter the conditions of the internal atmosphere by causing permeability distraction to carbon dioxide, oxygen, and water. This could lower transpiration loss, slow down the rate of transpiration, and postpone fruit ripening phase. According to Jianglian et al. (2013), the edible coating may stop moisture and scent loss, stop microbial growth, and stop oxygen from penetrating plant tissues.

7. METHODS OF APPLICATION OF CHITOSAN NANOPARTICLES

7.1 SEED TREATMENT

Chitosan is used as a film for seed, which helps deliver fertilizers, micronutrients, and plant protection products such as essential oils and others. It helps elicit systemic confrontation in the plants.

7.2 SOIL APPLICATION

Although chemical-based fertilizers and pesticides have high and immediate impacts on crop yield, they also negatively affect the environment and consumers. Less than 0.1% of agrochemicals are delivered to plant systems, and the rest are washed off into the atmosphere. Chitosan NPs are studied for their utilization in agriculture as a soil applicant.

7.3 FOLIAR APPLICATION

Foliar application of CNPs is used to increase the growth and production in the plant. Chitosan NPs get easily absorbed by leaves, penetrate the plant through stomata, travel down into the plant through the phloem, and provide nutrient to different parts.

8. CONCLUSION

Chitosan is a potential tool for innovative technologies that improve quality and environmental sustainability. Currently, the production of chitosan NPs has been delivered only on a laboratory scale. However, the transfer to large-scale production units followed by farmer's fields is still a huge challenge. Taking all points together in a frame, more research efforts are required to exploit the full potential of chitosan-based NPs in plants.

9. REFERENCES

1. Agnihotri, S. A., Mallikarjuna, N. N., &Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro and nanoparticles in drug delivery. *Journal of Controlled Release*, 100, 5-28.
2. Alfy, H., Ghareeb, R.Y., Soltan, E.L.S.A.Y.E.D., & Farag, D.A., 2020. Impact of chitosan nanoparticles as insecticide and nematicide against *Spodoptera littoralis*, *Locusta migratoria*, and *Meloidogyne incognita. Plant Cell Biotechnology Molecular Biology*, *21*, 126-140.
3. Al-Zahrani, S.S., Bora, R.S.,& Al-Garni, S.M., 2021. Antimicrobial activity of chitosan nanoparticles. *Biotechnology & Biotechnological Equipment*, 35(1), 1874-1880.
4. Atta, O. M, Manan S, Shahzad, A., Ul-Islam, M., Ullah, M. W., & Yang, G. (2022). Biobased materials for active food packaging: A review. *Food Hydrocolloids,*125, 107419.
5. Attwood, M. M.,& Zola, H. (1967). The association between chitin and protein in some chilinous tissues. *Comparitive Biochemistry and Physiology*, 20, 993.
6. Bashir, S.M., Ahmed Rather, G., Patrício, A., Haq, Z., Sheikh, A.A., Shah, M.Z.U.H., Singh, H., Khan, A.A., Imtiyaz, S., Ahmad, S.B., & Nabi, S., (2022). Chitosan nanoparticles: a versatile platform for biomedical applications. *Materials*, *15*(19), 6521.
7. Cacciatore, F. A., Brandelli, A.,& Malheiros, P. D. S. (2021). Combining natural antimicrobials and nanotechnology for disinfecting food surfaces and controlling microbial biofilm formation. *Critical Reviews in Food Science and Nutrition,* 61(22), 3771-3782.
8. Calvo, P., Remunan-Lopez, C., Vila-Jato, J. L., &Alonso, M. J. (1997). Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *Journal of Applied Polymeric Science,* 63 (1), 125- 132.
9. Chandrasekaran, M., Kim, K. D.,& Chun, S. C. (2020). Antibacterial activity of chitosan nanoparticles: A review. *Processes*, 8(9), 1173.
10. Choorit, W., Patthanamanee W., Manurakchinakorn, S. (2008). Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells. *Bioresource Technology,* 14,6168–6173.
11. Choudhary, R.C., Kumari, S., Kumaraswamy, R.V., Sharma, G., Kumar, A., Budhwar, S., Pal, A., Raliya, R., Biswas, P., &Saharan, V., (2019). Chitosan nanomaterials for smart delivery of bioactive compounds in agriculture. *Nanoscale Engineering in Agricultural Management* (124-139).
12. Das, S., Chakraborty, J., Chatterjee, S., & Kumar, H. (2018). Prospects of biosynthesized nanomaterials for the remediation of organic and inorganic environmental contaminants. *Environmental Science: Nano*, 5(12), 2784-2808.
13. Dhillon, G. S., Kaur, S., Brar, S. K., and Verma, M. 2013. Green synthesis approach: extraction of chitosan from fungus mycelium. *Critical Review in Biotechnology*, 33, 379 - 403.
14. Divya, K., Thampi, M., Vijayan, S., Shabanamol, S., &Jisha, M. S. (2022). Chitosan nanoparticles as a rice growth promoter: evaluation of biological activity. *Archieves of Microbiology*, 204(1), 1-11.
15. El Gamal, A.Y., Atia, M.M., Sayed, T.E., Abou-Zaid, M.I., &Tohamy, M.R., (2022). Antiviral activity of chitosan nanoparticles for controlling plant-infecting viruses. *South African Journal of Science*, *118*(1-2), 1-9.
16. Elshamy, M.T., Husseiny, S. M.,&Farroh, K. Y. (2019). Application of nano-chitosan NPK fertilizer on growth and productivity of potato plant. *Journal of Scientific Research in Science,* 36 (1),424-441.
17. Esyanti, R. R., Zaskia, H., &Amalia, A. (2019). Chitosan nanoparticle-based coating as post-harvest technology in banana. *TheJournal of Physiology,* 1204(1), 12109.
18. Gamage, A., Jayasinghe, N., Thiviya, P., Wasana, M. D., Merah, O., Madhujith, T., & Koduru, J. R. (2023). Recent Application prospects of chitosanbased composites for the metal contaminated wastewater treatment. *Polymers*, 15(6), 1453.

19. Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M., &Jellouli, K. (2014). Structural differences between chitin and chitosan extracted from three different marine sources. *International Journal of Biological Macromolecules*, 65: 298–306.
20. Hajizadeh, S. H., Dadashzadeh, R., Azizi, S., Mahdavinia, G.R. and Kaya, O. (2023). Effect of Chitosan nanoparticles on quality indices, metabolites, and vase life of *Rosa hybrida* cv. Black magic. *Chemical and Biological Technologies in Agriculture 10*(1), 12.
21. Hassanisaadi, M., Bonjar, G.H.S., Rahdar, A., Pandey, S., Hosseinipour, A.,&Abdolshahi, R. (2021). Environmentally safe biosynthesis of gold nanoparticles using plant water extracts. *Nanomaterials*, *11*(8),2033.
22. Hejjaji, E.M., Smith, A.M., &Morris, G.A., (2018). Evaluation of the mucoadhesive properties of chitosan nanoparticles prepared using different chitosan to tripolyphosphate (CS: TPP) ratios. *International Journal of Biological Macromolecules*, *120*, pp.1610-1617.
23. Hossain, M. S.,&Iqbal, A.(2016) Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits.*International Food Research Journal*, 23, 277- 83.
24. Ingle, P.U., Shende, S.S., Shingote, P.R., Mishra, S.S., Sarda, V., Wasule, D.L., Rajput, V.D., Minkina, T., Rai, M., Sushkova, S., &Mandzhieva, S. (2022). Chitosan nanoparticles (ChNPs): A versatile growth promoter in modern agricultural production. *Heliyon*, *8*(11).
25. Jha, R., &Mayanovic, R.A. (2023). A Review of the preparation, characterization, and applications of chitosan nanoparticles in nanomedicine. Nanomaterials, 13(8): 1302.
26. Jianglian D 2013 Application of Chitosan-Based Coating in Fruit and Vegetable Preservation: A Review.*Journal of Food Processing Technologies*, 4 (5–8).
27. Jonassen, H., Kjoniksen, A. L.,&Hiorth, M. 2012. Stability of chitosan nanoparticles crosslinked with tripolyphosphate. *Biomacromolecules,* 13 (11), 3747-3756
28. Kashyap, P. L., Kashyap, P. L., Xiang, X., & Heiden, P. A. (2015). Chitosan nanoparticle based delivery systems for sustainable agriculture. *International Journal of Biological Macromolecules*, 77, 36-51.
29. Khanafari, A., Marandi, R., &Sanatei, S. (2008). Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods.*Iranian Journal of Environmental Health Science & Engineering*, 5:19–24.
30. Muzzarelli, R. A. A., Tanfani, F., &Scarpini, G. (1980). Chelating, film-forming and coagulating ability of the chitosan-glucan complex from *Aspergillus niger*.*Biotechnology and Bioengineering* 22, 885–896.
31. Nawaz, A., Rehman, H.U., Usman, M., Wakeel, A., Shahid, M.S., Alam, S., Sanaullah, M., Atiq, M., &Farooq, M., (2023). Nanobiotechnology in crop stress management: an overview of novel applications. *Discover Nano,*18(1), 74.
32. Punia Bangar, S., Chaudhary, V., Thakur, N., Kajla, P., Kumar, M., & Trif, M. (2021). Natural antimicrobials as additives for edible food packaging applications: A review. *Foods*, 10(10), 2282.
33. Rojas-Grau, M. A., Soliva-Fortuny R., & Martin-Bellos, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. *Trends in Food Science & Technology*20, 438–47.
34. Saad, A. M., Alabdali, A. Y. M., Ebaid, M., Salama, E., El-Saadony, M. T., Selim, et al.(2022). Impact of green chitosan nanoparticles fabricated from shrimp processing waste as a source of nano nitrogen fertilizers on the yield quantity and quality of wheat (*Triticum aestivum* L.) cultivars. *Molecules*, 27(17), 5640.
35. Saleh, T. A., Mustaqeem, M., & Khaled, M. (2022). Water treatment technologies in removing heavy metal ions from wastewater: A review. *Environmental Nanotechnology, Monitoring & Management*, 17, 100617.24.
36. Sarraf, M., Vishwakarma, K., Kumar, V., Arif, N., Das, S., Johnson, R., et al. (2022). Metal/metalloid-based nanomaterials for plant abiotic stress tolerance: An overview of the mechanisms. *Plants,* 11(3), 316.
37. Sekhon, B. S. (2014). Nanotechnology in agri-food production: an overview. *Nanotechnology, Science and Applications*, 31-53.
38. Sharma, G., Prajapati, D., Devi, K.A., Pal, A., & Saharan, V. (2022). Chitosan nanomaterials for delivery of micronutrients in plants. In *Role of chitosan and chitosan-based nanomaterials in plant sciences* (239-253),(In Press).
39. Soltani, A., Ncibi, S., Djebbi, T., Sadraoui, I. A., Abada, M. B., Chargui, H., Hassine, K., Laabidi, A., Mahmoudi, H., Majdoub, H., & Ben-Jemâa, J. M. (2022). Microencapsulation of rosemary *Rosmarinus officinalis* essential oil by Chitosan-gum Arabic and its application for the control of two secondary pests of stored cereals.

40. Soni, A. T., Rookes, J. E.,& Arya, S. S. (2023). Chitosan nanoparticles as seed priming agents to alleviate salinity stress in rice (*Oryza sativa* L.) seedlings. *Polysaccharides*, 4(2), 129-141.
41. Stasinska-Jakubas, M., & Hawrylak-Nowak, B. (2022). Protective, biostimulating, and eliciting effects of chitosan and its derivatives on crop plants. *Molecules,* 27(9), 2801.
42. Tortella, G., Rubilar, O., Pieretti, J.C., Fincheira, P., de Melo Santana, B., Fernández-Baldo, M.A., Benavides-Mendoza, A., & Seabra, A.B., 2023. Nanoparticles as a promising strategy to mitigate biotic stress in agriculture. *Antibiotics*, 12(2), p.338.
43. Tsai, G. J., &Su, W. H. (1999). Antibacterial activity of shrimp chitosan against *Escherichia coli*.*Journal of Food Protection,* 62, 239–243.
44. Upadhyay, U., Sreedhar, I., Singh, S. A., Patel, C. M., & Anitha, K. L. (2021). Recent advances in heavy metal removal by chitosan-based adsorbents. *Carbohydrate Polymers*, 251, 117000.
45. Wang, A., Li, J., Al-Huqail, A. A., Al-Harbi, M. S., Ali, E. F., Wang, J., & Eissa, M. A. (2021). Mechanisms of chitosan nanoparticles in the regulation of cold stress resistance in banana plants. Nanomaterials, 11(10), 2670.
46. Wang, P., Li, L., Pang, X., Zhang, Y., Zhang, Y., Dong, W.F. and Yan, R., 2021. Chitosan-based carbon nanoparticles as a heavy metal indicator and for wastewater treatment. *RSC Advances*, 11(20), 12015-12021.
47. Yadav, M., Goswami, P., Paritosh, K., Kumar, M., Pareek, N., & Vivekanand, V. (2019). Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresources Bioprocessing*, 6(1), 1-20.
48. Yan, N., &Chen, X. (2015). Sustainability: don't waste seafood waste. *Nature*, 524 (7564): 155-157.
49. Zhang, Y., Zhao, M., Cheng, Q., Wang, C., Li, H., Han, X.,& Li, Z. (2021). Research progress of adsorption and removal of heavy metals by Chitosan and its derivatives: A review. *Chemosphere*, 279, 130927.
50. Zhao, L. M., Shi, L. E., Zhang, Z. L., Chen, J. M., Shi, D. D., Yang, J., et al. (2011) Preparation and application of chitosan nanoparticles and nanofibers.*Brazilian Journal of Chemical Engineering* 28, 353 - 362.