

Chitosan And Its Derivatives For Agriculture Applications: A Review

ABSTRACT

Chitosan is a semi-synthetic natural polysaccharide derived from the deacetylation of chitin, which is found in the exoskeletons of crustaceans and the cell walls of fungi. The primary attributes that characterize the chitosan biopolymer are its molecular weight and degree of deacetylation. It also has certain unique properties such as biocompatibility, nontoxicity, biodegradability and antimicrobial activity, making it a promising material for various applications, particularly in agriculture. These characteristics can be tuned and optimized depending on the intended application of the chitosan biopolymer. The conjugation of primarily three reactive functional groups in chitosan has led to the development of its many derivatives. The article discusses about the properties, derivatives and applications of chitosan by highlighting its use as a chelating agent, controlled-release fertilizer, plant biostimulant, antimicrobial agent and in post-harvest storage of fruits and vegetables. The paper emphasizes the potential of chitosan-based materials in sustainable agricultural practices, thereby reducing the dependence on synthetic non-degradable polymers.

Keywords: Chitosan(ChT), Chitin, chelation, amendment, biopolymer

1. INTRODUCTION

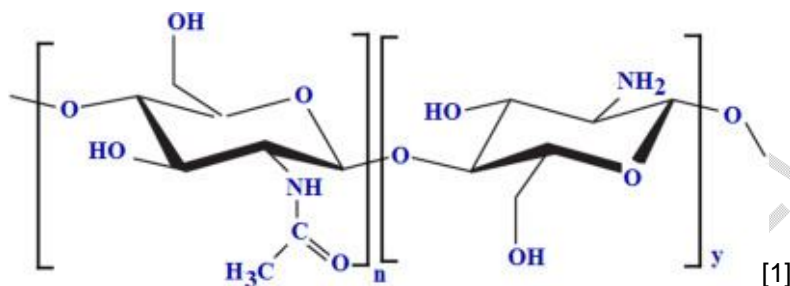
The majority of commercial polymers produced from petroleum-based source materials by processing chemistry are neither consistently safe or environmentally benign. Over the past three decades, there has been a growing interest in developing natural alternatives to synthetic polymers, namely biopolymers. Biopolymers are polymers derived from living organisms or synthesized from renewable resources. They have expanded significantly due to their biological origin and mostly to their non-toxicity, biocompatibility and biodegradability. Biopolymers include polysaccharides such as cellulose, starch, chitin/chitosan and alginates. Among these, one of the most prominent green polysaccharides that became popular in recent years is chitosan.

2. Chitosan

Chitosan is a semi-synthetic commercial amino polysaccharide derived by the deacetylation of the naturally occurring biopolymer called chitin. It was first discovered by Rouget in 1859, while studying deacetylated forms of chitin. But the term "chitosan" was coined by Hoppe-Seyler in 1894. The molecule consists of N-acetyl-2- amino-2-deoxy-D-glucopyranose (acetylated unit) and 2-amino-2-deoxy-D-glucopyranose (deacetylated unit), while the repeating units are linked by β -(1 \rightarrow 4) glycosidic bonds. Depending on the source of chitin, it occurs in three allomorphs viz. α , β and γ chitin [1]. Chitosan and chitin are characterized by their average degree of acetylation or degree of deacetylation and solubility

[2]. On the basis of the molecular weight or density of the polymeric chain, chitosan can be divided into two types viz. Low-Density Chitosan (LDC) and High-Density chitosan (HDC). The molecular weight and degree of deacetylation are the primary properties that characterize the chitosan biopolymer. Additional characteristics that are significant includes chelation, surface area, particle size, polycationic character and crystallinity. Understanding and controlling these properties is crucial for the effective utilization of this versatile material.

Image 1 : Structure of chitosan



3. Properties Of Chitosan

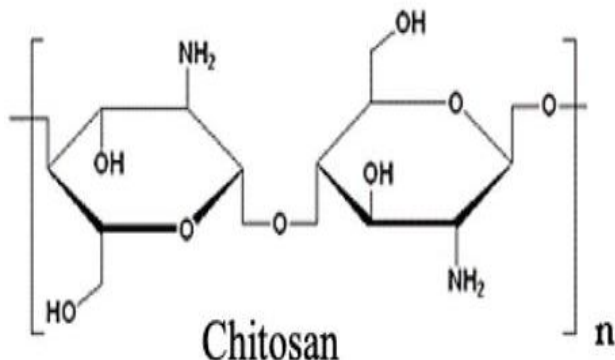
Chitosan possesses a rigid D-glucosamine structure with high crystallinity and hydrophilicity. Its deprotonated amino group acts as a powerful nucleophile (pKa 6.3) having capacity to form hydrogen bonds intermolecularly [3]. It has high viscosity, insoluble in water and organic solvents but soluble in dilute aqueous acidic solutions. Crosslinking of chitosan forms salts with organic and inorganic acids [4]. Chitosan possesses chelating and complex formation properties, high ionic conductivity and has polyelectrolytic properties. It is a cationic biopolymer that interacts with negatively charged molecules and has a high charge density (one positive charge per glucosamine residue) [5]. In order to isolate biomolecules, the molecule also has adhesion, filtration, separation, film-forming ability and trapping and adsorption capabilities. Important biological features are biocompatibility, nontoxicity, biodegradability, absorbability, bioactivity and antimicrobial property. Its biological properties are closely related to its physicochemical structure, which includes the degree of deacetylation, water content, and molecular weight [6].

4. Significance of Chitin

Chitin is considered as the second most abundant naturally occurring polysaccharide after cellulose that is rich in nitrogen (9-12%) than cellulose (1.25%) [7]. It is structurally poly[b-(1-4)-N-acetyl]-D-glucosamine. The structure of chitin ($C_8H_{13}O_5N$)_n is similar to that of cellulose, but with 2-acetamido-2-deoxy-β-D-glucose (NAG) monomer units, which are attached to each other via β(1→4) linkages [8]. Chitin occurs as crystalline microfibrils in nature, that form structural elements of the exoskeletons of arthropods and in fungal cell walls. Chitin has been existed mainly in three solid state polymorphic forms viz. α, β, and γ [9]. Out of these allomorphs, α-chitin is the most abundant one followed by β and then rarest one is γ-chitin. These forms differ in their orientation of microfibrils. In α-chitin, it consists of two N, N'-diacetylchitobiose units forming two chains antiparallel to each other with intersheet H bonds while in β-chitin the chains are parallel to each other without intersheet H bonds. γ-Chitin is the third allomorph, possessing mixed parallel and antiparallel orientations [10].

Image 2 :
Chitin

Structure of



5. Comparison of chitin and chitosan

Chitosan and chitin are known as copolymers of D-glucosamine and N-acetyl-D-glucosamine units which are characterized by their average degree of acetylation or deacetylation and solubility. When the number of D-glucosamine (deacetylated) units is higher than 50%, the biopolymer is termed as chitosan and vice versa [11]. Chitin and chitosan can also be defined on the basis of their differences in water solubility. Chitosan is soluble in aqueous acetic acid while chitin is insoluble and also in the vast majority of common solvents [12].

6. Various sources of chitin

Different sources for extraction and production of chitin can be categorized as:

6.1. Terrestrial Organisms:

Terrestrial species generally includes crustaceans such as *Porcellio scaber*, *Armadillidium vulgare*, arthropods, nematodes, insects, silkworms, mosquitoes, honeybee, *Sipylloidea sipylus*, *Drosophila melanogaster*, *Extatosoma tiaratum*, and many more

6.2. Marine or Aquatic Organisms:

Chitin produced from aquatic species includes diatoms, algae, crabs, shrimps, lobster and squids. The waste streams of molluscs and crustaceans are the main source of chitin. Also, they constitute a rich source of proteins, flavor compounds, and various pigments and hence are of great attention for different research fields and industries as well [13].

6.3. Microorganisms:

Microbial world mainly includes fungi (cell wall, mycelia, septa) molds, chrysophyte algae, yeasts, prosthecate bacteria, spores of streptomycete and ciliates. Except Oomycetes, remaining Ascomycetes, Basidiomycetes, Deuteromycetes, and Zygomycetes consists of 2–65% chitin/chitosan [14].

7. Method Of Chitosan Preparation

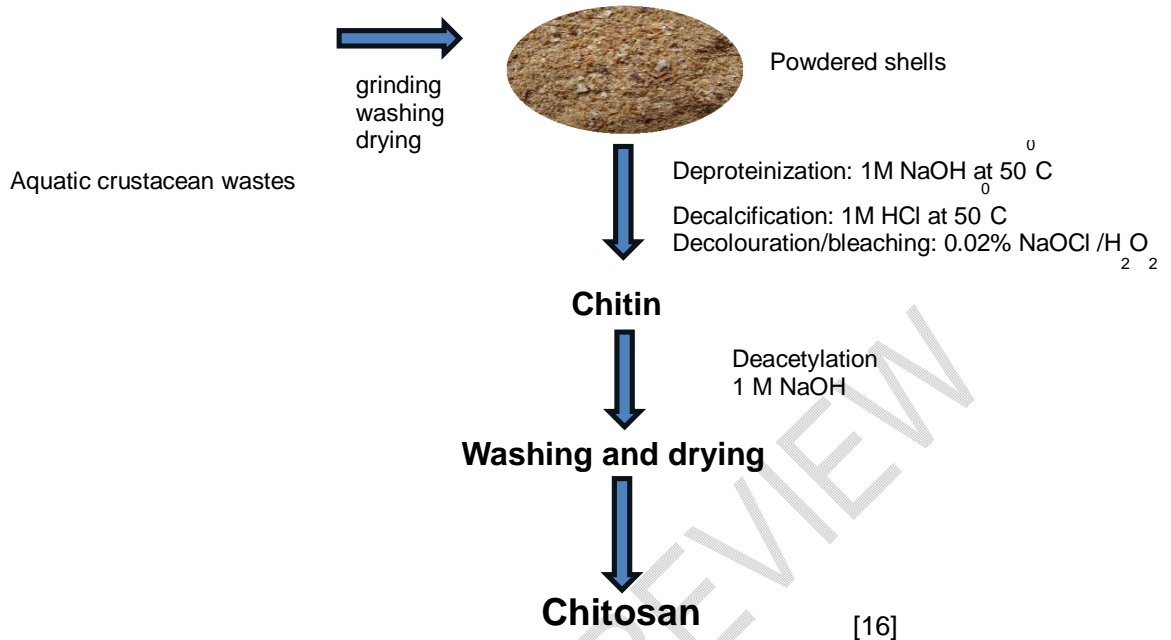
Based on the origin of chitin sources, the preparation of chitosan consists of two methods namely, deacetylation and fermentation method.

7.1. Deacetylation method

Chitosan is derived from chitin through deacetylation process whereby, the acetamide group is hydrolysed to produce acetate ions and an $-NH_2$ group. Production of chitosan from raw shells involves four major steps, viz., deproteination, demineralization, decolourisation and deacetylation [15]. Raw materials most abundantly available for chitin production are the shells of crab, shrimps and prawn (69- 70%) and is associated with other constituents where chemical treatments are needed for removing impurities. Trypsin, papain and other proteolytic enzymes can be used in digestion to eliminate proteins as can sodium hydroxide. On the other hand, Hydrochloric acid is used to remove minerals such calcium phosphate and carbonate. Conversion of chitin to chitosan generally is achieved by the hydrolysis of acetamide groups of chitin by severe alkaline hydrolysis treatment. It is due to the resistance of such groups imposed by the trans-arrangement of the C2-C3 substituent in the sugar ring [16].

Chart 1 : Deacetylation method



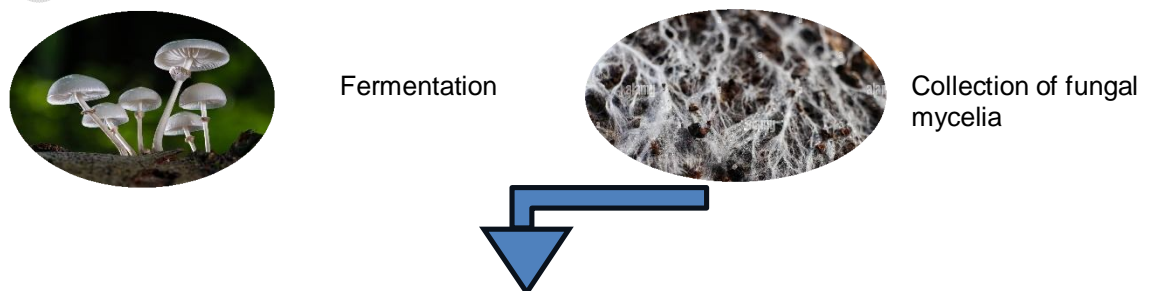


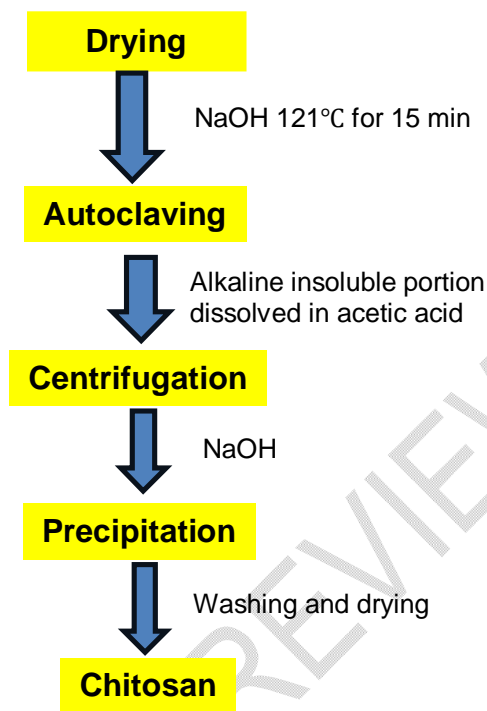
7.2. Fermentation Method

Fungal mycelia have lower level of inorganic materials compared to crustacean waste and thus no demineralisation step is required here. The fungi exposed to fermentation in order to collect the fungal mycelia followed by alkaline treatment to remove the protein and alkali soluble polysaccharides are removed with 1M NaOH at 12°C for 15 minutes which is followed by centrifugation and precipitation with NaOH that result in the production of chitosan [17].

By comparing the two methods, chitosan produced by fungus is of high quality than chitosan produced by crustaceans due to degree of deacetylation, molecular weight, viscosity and charge distribution of the fungal chitosan. They are more stable than crustacean chitosan. The fungal chitosan is free of heavy metal contents such as nickel and copper [18]. Moreover, the production of chitosan from fungal mycelia gives medium-low molecular weight chitosan (1×10^4 Da), whereas from crustacean sources is of high molecular weight (about 1.5×10^6 Da). For these reasons, there is an increasing interest in the economic production of fungal chitosan [19].

Chart 2 : Fermentation Method





[16]

8. Derivatives Of Chitosan

Chitosan can easily be modified by chemical processes to prepare chitosan derivatives and these processes may be used for controlling the reactivity of the polymer that include improving the affinity of the sorbent for the metal, changing the selectivity series for sorption, or by changing the pH to increase the chemical stability[20]. Chitosan has three types of reactive functional groups: two hydroxyl groups on C-3 and C-6 in each repeating unit and one amino group on C-2 in each deacetylated unit. These groups allow the conjugation of many substituents resulting in newly modified derivatives. The C3- OH has large spatial site resistance and is relatively difficult to modify while C2- NH₂ is highly reactive for fine modifications and is the most commonly used modifying group in chitosan. However, the low bioactivity and solubility of chitosan at physiological pH condition (greater than pH 6.5) certainly limits the application of chitosan. Therefore, reasonable modifications of chitosan are needed to obtain derivatives with increased activity and improved water solubility. The main reactions are acetylation, reductive amination, amidation, acylation, phosphorylation and crosslinking modifications [21].

Among these reactions, the most frequently used reactions are (i) formation of Schiff bases (reductive amination) and (ii) carbodiimide-mediated amidation. Reductive amination refers to the formation of imine products between amine and carbonylic (aldehydes and ketones) groups. This reaction is very simple and takes place under mild conditions. The imine could be easily reduced with a suitable reducing agent like sodium borohydride (or, preferably, sodium cyanoborohydride) that produce selective N-alkyl derivatives. The carbodiimide-mediated amidation involves a group of activators for the amidation of amines with carboxylic groups. When using these reagents, the amidation is straightforward and usually takes place under mild conditions at room temperature. Most frequently used activators are N-dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3-dimethylamino) propyl) carbodiimide (EDC) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). In order to increase yields and decrease side reactions, N-hydroxy succinimide (NHS) is often added [22].

9. Chitosan- a superior chelating agent

Chelation is a type of bonding between ions and molecules to a metal ion that involves formation of two or more separate coordinate bonds between a multidentate ligand and an individual central atom. Chitosan was found to have the highest chelating ability in comparison with other natural polymers due to the presence of $-NH_2$ and $-OH$ groups that are the main reaction sites involved in chelate formation [23]. The degree of polymerization, deacetylation and the distribution of acetyl groups along the polymer chain are of crucial importance for chitosan metal interacting characteristics. The phenomenon of chelation depends on the number of empty molecular orbitals and usually occur at nearly neutral pH [24]. The mechanism of complex formation between chitosan and metal ions during adsorption process can be developed in two ways. They are, (a) Bridge model and (b) Pendant model. In bridge model, metal ions are bonded with various amino groups from the same chain or from different chains through complex inter- or intramolecular reactions whereas in pendant model, metal ions are bonded with amino groups in a hanging manner.

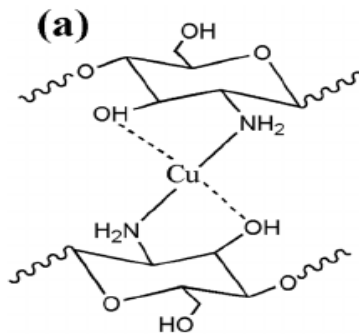


Fig.1. Bridge model

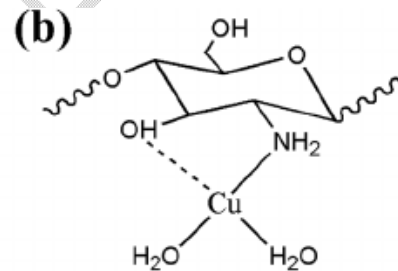


Fig.2. pendant model

9.1 Factors affecting chelation

9.1.1 pH effect: pH change leads to an alteration in the metal speciation, the number of protonated amino groups and consequently in metal ion adsorption capacity. At low pH, protonation of amino sites decreases the chelating capacity and near to neutral pH is the more favorable for efficient chelate formation. Also, at higher pH conditions metal chelation has been diminished due to rapid change in protonated and unprotonated forms of chitosan [25].

9.1.2 Ion Selectivity: Chitosan has neither affinity for alkaline and alkaline-earth metals nor is it selective for transition metals. Affinity of chitosan for cations doesn't depend on the physical form of the polymer, and that this selectivity does not depend on the size and hardness of considered ions. The adsorption of ions mixtures, showed the selectivity rule as follows: $Cu(II) \geq Hg(II) > Zn(II) > Cd(II) > Ni(II) \geq Co(II)$ [26].

9.1.3 Deacetylation degree: The fraction of deacetylation is the major parameter to influence complexation since it directly affects the density of amino groups on the surface of chitosan and, therefore, the capacity and mechanism of complex formation [27].

10. Application of chitosan in agriculture

Chitosan is an active molecule that finds many possible applications in agriculture with the aim of reducing or replacing more environmentally damaging chemical substances. It is considered as a prominent biopolymer in the field of agriculture that helps in application as controlled release fertilizer, plant stimulant, plant growth promoter, plant disease suppressor, antimicrobial property, alleviates biotic and abiotic stress, use in seed treatments, as soil amendment, as a biofertiliser, insecticides, effects on post-harvest fruits and vegetables etc.[28].

10.1 Chitosan- as a controlled release fertiliser (CRF)

Fertilisers are crucially important for delivering nutrients to plants for producing high-quality crops for food security [29]. At the same time, they are also identified as agricultural pollutants as they cause damages to biodiversity with intensifying influences due to altering consumption patterns and growing inhabitants by transforming natural habitats to extremely sophisticated arrangements and discharging pollutants and greenhouse gases. Therefore, numerous studies have been conducted for uniform nutrient distribution using naturally occurring degradable materials and rapidly developing smart nanomaterials by using biopolymers that can contribute a vital role in developing ecofriendly sustainable agricultural activities [30]. Recently, the application of chitosan in controlled-release fertiliser is still in the primary stage. However, it can be an excellent fertiliser based on its biodegradability and eco-friendly character and also due to its porous structure on the surface which is conducive to water and nutrient permeation [31].

10.1.1 Preparation of chitosan based CRF

The controlled-release fertilisers developed by chitosan hydrogels possess a diffusion process that controls the chemical kinetics and water absorption [32]. The emulsification-cum-cross-linking technique is an exemplary method for synthesizing CRF. [33]. At first the chitosan powder gets mixed with aqueous solution of lactic acid followed by mechanical stirring. Then the potato starch gel was prepared by heating 8% (wt/v) starch (ST) solution with deionized water with constant magnetic stirring. Later gelatinization was achieved at 76 °C in a boiling distilled water bath. The cross-linking solution was prepared by dissolving sodium tripolyphosphate (TPP) which act as an emulsifying agent in distilled water to produce a final concentration of 1% (wt/v) at pH 8.6. Dry polymeric matrices were immersed in the fertiliser-saturated aqueous solution for 4 hr. at room temperature. After the swelling process, the microspheres were dried at 40 °C for 48 hr and used as CRF [34]. Studies has showed that encapsulation of urea into salicyl-imine-chitosan hydrogels by cross linking has enhanced water absorbency of soil up to 154 percent and resulted in slow release of urea that has enhanced the growth and fruit quality in tomato [35].

10.1.1.1. Nutrient transporter method of delivery with chitosan-based nanoparticles

Chitosan in the form of nano particles act as a carrier to provide the optimum amount of nutrients to the plants. Chitosan will increase the plant metal uptake by trapping and thereby releasing them slowly so that they become available for plant use in a timely manner. The major advantages of encapsulating agrochemicals in a chitosan matrix as nanoparticles include its ability to function as a protective reservoir for the active ingredients thereby protecting the ingredients from the surrounding environment

while they are in the chitosan domain and then controlling their release and allowing them to serve as efficient nutrient delivery systems for plant transformation [36].

10.2. Chitosan – as a supplement for soil

Chitosan has the ability to alleviate soil fertility. It possesses higher nitrogen content (9-12%) as compared to other chitin products. Chitosan also contains substantial levels of calcium minerals, which provide structural rigidity to soil. The cationic properties of chitosan also make it suitable as a medium for supplying additional essential nutrients. The functional hydroxyl and amino groups on deacetylated chitin (chitosan) allow the formation of coordination compounds with ions of copper, zinc, iron and other micronutrients that enhance the soil fertility. Due to its high molecular weight and complexity in structure, chitosan can form gels that absorb substantial volumes of water to increase soil water holding capacity. Plant growth stimulation by chitosan as a soil amendment might also result from its direct effect on plant nutrient status, metabolism, photosynthesis rate, increase in nitrogen, phosphorus, potassium, total sugars and soluble proteins as well as total amino acids [37]. Soil application of chitosan is more complex than foliar spraying that includes (a) increased bioavailability of nitrogen due to the high content of chitin and the low C/N ratio, (b) increased activity of chitinolytic organisms that causes antagonistic effects against plant soil pathogens and (c) the favorable effects on soil microbiota such as mycorrhiza and rhizobia that act synergistically to plant and improve crop performance [38].

10.3. Chitosan for seed treatments

The use of chitosan on seeds as film coating delivery system act as a physical barrier against the attack of pest and disease. It also enhances seeds germination index, reduce the mean germination time and flowering time along with an increase in plant growth and biomass. The observed growth improvement by chitosan could be also related to the incorporation of nutrients, particularly nitrogen. Application of chitosan on wheat seeds against *Fusarium graminearum* showed increased content of phenols, increased activities of defense related enzymes and antioxidants that resulted in better growth and development against the pathogen [39]. Another study of exogenous priming action of chitosan has induced the upregulation of phytohormones and resistance against powdery mildew in cucumber. The seed germination and vigour index were registered maximum with optimum concentration of chitosan through seed priming [40].

10.4. Chitosan- plant disease suppressor

Chitosan polymer (CHT) shows an effective antimicrobial activity against a wide range of micro-organisms, including fungi, bacteria, viruses and nematodes. The electrostatic interaction of negatively charged cell membrane of microbes with positively charged chitosan and its derivative leads to agglutination, destruction and alteration of cell membrane structure that leads to death of microorganisms [41].

10.5. Microbial activity of chitosan

Chitosan's microbial activity involves a variety of mechanisms to combat different bacterial, fungal, and viral diseases.

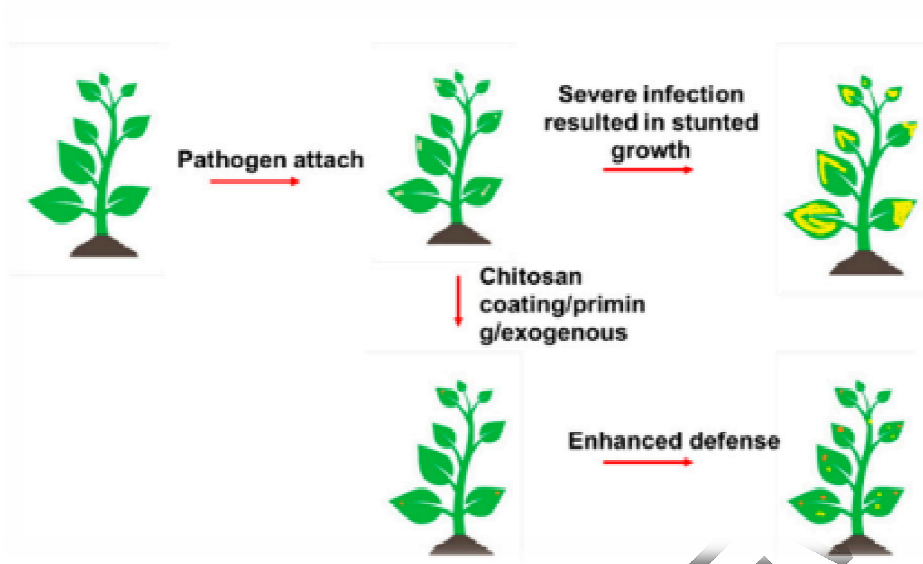


Fig.3. Mechanism of microbial activity by chitosan

[16]

10.5.1. Antifungal activity of chitosan biopolymer

Chitosan prevents the growth of several harmful pathogenic fungi in vitro, such as *Alternaria alternata*, *Botrytis cinerea*, *Penicillium digitatum* etc. It involves in the growth suppression of pathogen that causes inhibition on hyphal growth, spore formation, spore viability, germination and fungal virulence factor production [42].

10.5.2. Antibacterial activity of chitosan

Chitosan biopolymer has strong in vitro and in vivo antibacterial activities towards different plant pathogenic bacteria, like *Xanthomonas spp.*, *Pseudomonas spp.*, *Streptomyces scabies*, *Burkholderia seminalis* etc. The inhibitory activity of CHT against bacteria varied with concentration of CHT used, molecular weight, bacterial type (Gram-positive and Gram-negative), bacterial surface, cell wall composition structure, solvent type, period of incubation and abiotic factors [43].

10.5.3. Antiviral activity of chitosan

Chitosan has been demonstrated to suppress a few plants viral diseases. In recent studies it has showed tremendous medicinal affect for tackling COVID-19 in humans and further researches are still going on [44]. It inhibits the systemic proliferation of viroids and viruses and enhances the host's hypersensitive reactions to infection. Chitosan ability to inhibit viral plant infections is not dependent on type of virus, as CHT impacts the plant itself by triggering resistance to viral diseases [45].

10.6. Nematicidal activity of CHT

Application of chitosan biopolymer in the soil promotes the multiplication of chitinolytic microorganisms that degrades chitin containing the organ of plant parasitic nematodes thereby reduces egg hatching and the viability of larvae and adults belonging to *Meloidogyne sp.* thereby ameliorate the micro-environment to promote the growth of nematophagous bacteria and fungi. The nematocidal activity has increased the ammonia concentration due to chitin hydrolysis. It has also stimulated the growth of bacteria, actinomycetes and a limited number of fungal

species with chitinolytic properties as antagonistic microorganisms [46]. It was also found that application of chitosan (2 mg/L) significantly increased the egg parasitisation of *M. javanica* by nematophageous and endophytic fungus *Pochonia chlamydosporia* by increasing the activity of aryl phosphatase, chitinase and urease in soil [47].

10.7. Effect of chitosan on biotic stress

Under biotic stress, plants exhibit defense response including production of phytoalexin, pathogenesis-related proteins such as chitinase and β -glucanase, proteinase inhibitors, callose formation, lignin biosynthesis and induction of stress-responsive genes. However, upon treatment with chitosan and its oligomers, these defense-related compounds are found to increase, facilitating the use of chitosan and its derivatives as strong antimicrobial compounds and elicitors for plant protection. Chitosan also induces the production of jasmonic acid (JA) accumulation in plants. Jasmonic acid and ABA have similar activities which play a significant role in the regulation of water use by plants [48].

10.8. Effect of chitosan on abiotic stress

Chitosan showed significant effect on abiotic factors like drought, salinity stress and heavy metal toxicity. During drought condition foliar application of chitosan enhances the production of metabolites and amino acids and inhibits the secretion of MDA (malonaldehyde) and lipid peroxidation thereby improved membrane stability. Chitosan treatment on plants grown in salt affected soil result in increased stomatal conductance, photosynthetic rate, internal CO₂ concentration and antioxidant enzyme activity. Chitosan form complexes with heavy metals due to the presence of functional amino and hydroxyl group and alleviate toxicity of cadmium due to its porous structure and presence of NH₂ and OH group [49].

10.9. Chitosan effects on post-harvest fruits and vegetables

The diverse beneficial effects of chitosan in fruit shows great value. Coating of post-harvest fruits with edible chitosan showed a reduction in the percentage of rotten tissue, increase in ascorbic acid content, increase in shelf-life, enhance the antioxidant activity and Vit C and maintain freshness of the fruits [50].

10.10. Chitosan as a Bio-Fertilizer and Fertilizer Protectant

Chitosan as a biofertiliser degrade enzymatically without affecting the soil-borne beneficial rhizosphere biota and also induce the symbiotic exchange between plant and microbes [51]. In addition, chitosan is a polysaccharide-based biopolymer, which stimulates the activity of plant symbiotic microbes, resulting in the alteration of rhizosphere microbial equilibrium, thus disadvantaging the plant pathogens [52].

11. Disadvantages of chitosan

Chitosan possesses certain limitations like low surface area, poor solubility, resistant to acidic medium and poor thermal conductivity due to its complex structure and molecular weight [53]. So, the use of chitosan and its derivatives depends upon the field of application, molecular weight, dosage and the degree of deacetylation.

12. Conclusion

Chitosan is one of the most important derivatives of chitin, which is the second most abundant natural biopolymer found on earth after cellulose and is a major component of the shells of crustaceans such as crabs and shrimps. It can be obtained by N deacetylation of chitin and it is a co-polymer of glucosamine and N-acetylglucosamine units linked by 1–4 glycosidic bonds and was found to have the highest chelating ability in comparison with other natural polymers. The main characteristics of chitosan are hydrophilicity, harmlessness for living things and biodegradability, easy chemical derivatization and capability to adsorb a number of metal ions. The use of chitosan in agriculture is fetching great attention in various crop improvement and crop protection fields. Therefore, there is more scope in the development of more derivatives of chitosan through novel technologies thereby contributing towards sustainable agriculture practices by reducing more dependence on synthetic non-degradable polymers.

13. REFERENCES

1. Elieh, AKD, Hamblin MR. Chitin and chitosan: production and application of versatile biomedical nanomaterials. *International journal of Advanced Research*. 2016. 4(3): 400-411.
2. Crini, NM, Lichtfouse F, Torri G, Crini G. Fundamentals and applications of chitosan. In: Crini, G. and Lichtfouse, E. (eds), *Scrivener publishing, Sustainable agriculture reviews*. 2019. 35: 394-401.
3. Rinaudo M. Chitin and chitosan: Properties and applications. *Progress in polymer science*. 2006 Jul 1;31(7):603-32.
4. Badawy ME, Rabea I and Entsar I. A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection. 2011. *International Journal of Carbohydrate Chemistry*. 20(5): 1-29.
5. Rudall KM, Kenchington W. The chitin system. *Biological Reviews*. 1973; 40:597–636
6. Zhang M, Zhang F, Li C., Ann H, Wan T, Zhang P. Application of Chitosan and Its Derivative Polymers in Clinical Medicine and Agriculture. *Polymer*. 2022. 14(5): 950-958.
7. Jolanta KW, Thoming J, Stepnowski P. Biomedical Activity of Chitin/ Chitosan Based Materials-Influence of Physicochemical Properties Apart from Molecular Weight and Degree of N-Acetylation. *Polymers*. 2011.52(8): 259-266.
8. Yu C, Kecen X, Xiaosai Q. Grafting modification of chitosan. In *Biopolymer Grafting*. 2018;295-364
9. Atkins ED, Dlugosz J, Foord S. Electron diffraction and electron microscopy of crystalline α -chitin from the grasping spines of the marine worm *Sagitta*. *International Journal of Biological Macromolecules*. 1979;1(1):29-32.
10. Minke R, Blackwell J. The structure of α -chitin. *Journal of Molecular Biology*. 1978; 120:167–81.

11. Arbia W, Arbia L, Adour L, Amrane A. Chitin extraction from crustacean shells using biological methods—a review. *Food Technology and Biotechnology*. 2013;51(1):12-25.
12. Crini G, Gimbert F, Robert C, Martel B, Adam O, Morin-Crini N, De Giorgi F, Badot PM. The removal of Basic Blue 3 from aqueous solutions by chitosan-based adsorbent: Batch studies. *Journal of Hazardous Materials*. 2008;153(1-2):96-106.
13. Iber BT, Kasan NA, Torsabo D, Omuwa JW. A review of various sources of chitin and chitosan in nature. *Journal of Renewable Materials*. 2022;10(4):1097.
14. Annu AS, Ikram S. Chitin and chitosan: History, composition and properties. In: Ahmed, S. and Ikram, S. (eds), *Chitosan derivatives, composites and application*. 2.17. pp. 3-24.
15. Kurita T, Tomita K, Tada T, Nishimura SI, Ishii S. Reactivity characteristics of a new form of chitosan. *Polymer Bulletin*. 1993. 30: 429–433.
16. Pandey P, Verma MK, De N. Chitosan in agricultural context—a review. *Bulletin of Environment, Pharmacology and Life Science*. 2018.7(4):87-96.
17. Winterowd, Jack G, Paul A. Sandford. "Chitin and chitosan." *Food Science And Technology-New York-Marcel Dekker*. 1995: 441-441.
18. Crestini C, Kovac B, Giovannozzi G. Production and isolation of chitosan by submerged and solid-state fermentation from *Lentis nusedodes*. *Biotechnology Bioengineering*. 1996. 50: 207–210.
19. Sandford PA. Advances in chitin science. *Carbohydrate Polymer*. 2018. 56(1): 59-95.
20. Guibal E. Interactions of metal ions with chitosan-based sorbents: a review. *Separation and purification technology*. 2004. 38(1):43-74.
21. Arguelles-Monal WM, Lizardi-Mendoza J, Fernández-Quiroz D, Recillas-Mota MT, Montiel-Herrera M. Chitosan derivatives: introducing new functionalities with a controlled molecular architecture for innovative materials. *Polymers*. 2018. 20;10(3):342.
22. Sahariah P, Másson M. Antimicrobial chitosan and chitosan derivatives: a review of the structure–activity relationship. *Biomacromolecules*. 2017;18(11):3846-68.
23. Varma AJ, Deshpande SV, Kennedy JF. Metal complexation by chitosan and its derivatives: a review. *Carbohydrate polymers*. 2004;55(1):77-93.
24. Nechita P. Applications of chitosan in wastewater treatment. *Biological activities and application of marine polysaccharides*. 2017;(5395720):209-28.
25. Machado BR, Roberto SB, Bonafe EG, Camargo SE, Camargo CH, Popat KC, Kipper MJ, Martins AF. Chitosan imparts better biological properties for poly (ϵ -caprolactone) electro spun membranes than dexamethasone. *Journal of the Brazilian Chemical Society*. 2019; 30:1741-50.

26. de Farias BS, Grundmann DD, Rizzi FZ, Martins NS, Junior TR, de Almeida Pinto LA. Production of low molecular weight chitosan by acid and oxidative pathways: Effect on physicochemical properties. *Food Research International*. 2019;123:88-94.
27. Bandara S, Du H, Carson L, Bradford D, Kommalapati R. Agricultural and biomedical applications of chitosan-based nanomaterials. *Nanomaterials*. 2016;10(10):1903.
28. Chakraborty M, Hasanuzzaman M, Rahman M, Khan MA, Bhowmik P, Mahmud NU, Tanveer M, Islam T. Mechanism of plant growth promotion and disease suppression by chitosan biopolymer. *Agriculture*. 2020;10(12):624.
29. Kusumastuti Y, Istiani A, Rochmadi, Purnomo CW. Chitosan-based Polyion multilayer coating on NPK fertilizer as controlled released fertilizer. *Advances in Materials Science and Engineering*. 2019;2958021.
30. Kumar S, Nehra M, Dilbaghi N, Marrazza G, Hassan AA, Kim KH. Nano-based smart pesticide formulations: Emerging opportunities for agriculture. *Journal of Controlled Release*. 2019; 294:131-53.
31. Kalia A, Sharma SP, Kaur H, Kaur H. Novel nanocomposite-based controlled-release fertilizer and pesticide formulations: Prospects and challenges. Multifunctional hybrid nanomaterials for sustainable agri-food and ecosystems. 2020;99-134.
32. Corradini E, De Moura MR, Mattoso LH. A preliminary study of the incorporation of NPK fertilizer into chitosan nanoparticles. *Express polymer letters*. 2010;4(8).
33. Wang J, Liu S, Qin Y, Chen X, Xing RE, Yu H, Li K, Li P. Preparation and characterization of controlled-release fertilizers coated with marine polysaccharide derivatives. *Chinese Journal of Oceanology and Limnology*. 2017;35(5):1086-93.
34. Perez JJ, Francois NJ. Chitosan-starch beads prepared by ionotropic gelation as potential matrices for controlled release of fertilizers. *Carbohydrate polymers*. 2016; 148:134-42.
35. Iftime MM, Ailiesei GL, Ungureanu E, Marin L. Designing chitosan based eco-friendly multifunctional soil conditioner systems with urea-controlled release and water retention. *Carbohydrate polymers*. 2019. 223:115040.
36. Mohamed AK, Ahmed HM, Mohamed AM, Moustafa AA. Effect of spraying chitosan on growth, yield and berries quality of Flame Seedless grapevines. In 10th International Conference for Sustainable Agricultural Development, Fayoum *Journal of Agricultural Research and Development*. 2020; 34:16-27.
37. Xu C, Mou B. Chitosan as soil amendment affects lettuce growth, photochemical efficiency, and gas exchange. *Horticultural Technology*. 2018;28(4):476-80.

38. Shahrajabian MH, Chaski C, Polyzos N, Tzortzakis N, Petropoulos SA. Sustainable agriculture systems in vegetable production using chitin and chitosan as plant biostimulants. *Biomolecules*. 2021;11(6):819.
39. Orzali L, Corsi B, Forni C, Riccioni L. Chitosan in agriculture: a new challenge for managing plant disease. *Biological activities and application of marine polysaccharides*. 2017; 10:17-36.
40. Jogaiah S, Satapute P, De Britto S, Konappa N, Udayashankar AC. Exogenous priming of chitosan induces upregulation of phytohormones and resistance against cucumber powdery mildew disease is correlated with localized biosynthesis of defense enzymes. *International Journal of Biological Macromolecules*. 2020;162:1825-38.
41. Sarmiento B, Goycoolea FM, Sosnik A, das Neves J. Chitosan and Chitosan Derivatives for Biological Applications: Chemistry and Functionalization. *International Journal of Carbohydrate Chemistry*. 2011;19-22.
42. Badawy ME, Rabea EI, Eid AR, Badr MM, Marei GI. Structure and antimicrobial comparison between N-(benzyl) chitosan derivatives and N-(benzyl) chitosan tripolyphosphate nanoparticles against bacteria, fungi, and yeast. *International Journal of Biological Macromolecules*. 2021; 186:724-34.
43. Farag ZS, Sharaf AM, Morsy MK. Influence of chitosan-based coating incorporation green tea and rosemary extracts on physicochemical and microbial quality of Tilapia fish (*Oreochromis niloticus*) fillets under cold storage. *Egyptian Journal of Food Science*. 2018; 46:69-82.
44. Safarzadeh M, Sadeghi S, Azizi M, Rastegari-Pouyani M, Pouriran R, Hoseini MH. Chitin and chitosan as tools to combat COVID-19: A triple approach. *International journal of biological macromolecules*. 2021; 183:235-44.
45. Badawy ME, Rabea EI. Chitosan and its derivatives as active ingredients against plant pests and diseases. In *Chitosan in the preservation of agricultural commodities* 2016; 179-219.
46. Fan Z, Qin Y, Liu S, Xing R, Yu H, Li P. Chitosan oligosaccharide fluorinated derivative control root-knot nematode (*Meloidogyne incognita*) disease based on the multi-efficacy strategy. *Marine Drugs*. 2020;18(5):273.
47. Archana NH, Rani OP. Potential of the Natural Biopolymers, Chitin and Chitosan in Root-Knot Nematode *Meloidogyne incognita* (Kofoid and White) Chitwood Management. *Indian Journal of Nematology*. 2018;6-12.
48. Katiyar D, Hemantaranjan A, Singh B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology*. 2015; 20:1-9.
49. Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. Application of chitosan on plant responses with special reference to abiotic stress. *Physiology and molecular biology of plants*. 2019; 25:313-26.

50. Sharif R, Mujtaba M, Ur Rahman M, Shalmani A, Ahmad H, Anwar T, Tianchan D, Wang X. The multifunctional role of chitosan in horticultural crops; a review. *Molecules*. 2018;23(4):872.
51. Petropoulos SA. Practical applications of plant biostimulants in greenhouse vegetable crop production. *Agronomy*. 2020;10(10):1569.
52. Agbodjato NA, Noumavo PA, Adjanooun A, Agbessi L, Baba-Moussa L. Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (*Zea mays L.*). *Biotechnology research international*. 2016;(1):7830182.
53. Badawy ME, Rabea EI. Chemical Modification of Chitin and Chitosan for Their Potential Applications. In *Industrial Applications of Marine Biopolymers* 2017;117-175.

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