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Chitosan-An Antimicrobial Agent and A Plant Resistance Inducer

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Abstract
Chitosan (CS) is a natural biopolymer derived from alkaline deacetylation of chitin of crustaceans and fungal cell walls. It has exceptional biomedical, antimicrobial, antitumor and eco-friendly properties. Unusual application of toxic chemical pesticides for crop protection has led to environmental contamination and human health damage. In recent years CS is using as an alternative to chemical pesticides to control the plant diseases. Its application is simple, inexpensive and safe to the environment. In agriculture it is using in seed treatment and foliar application on plants to avoid microbial infections. Further, many studies have reported that CS acts as a potential elicitor for induction of disease resistance in plants against many pathogens. Here we focused to explore antimicrobial and plant resistance inducing natures of CS as to protect plants from various plant diseases.

Keywords
Chitosan, antimicrobial activity, elicitors, defense mechanism and plant resistance.

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1. INTRODUCTION
In agriculture, various economically important crops are being infected by several plant pathogens and account for huge crop loss around the world. For the past few decades, in order to control plant diseases and to improve crop productivity, limitless employ of chemical pesticides and fertilizers has been done, which has eventually led negative impact on ecosystem through soil and water contamination and entering into consumers food chain [1-2].

Several studies have focused to develop alternatives to chemical pesticides mediated control of crop diseases [3]. Application of natural components of plant and animal extracts has shown promising impact on control of plant pathogens and inducing plant resistance. Plant extracts (monoterpenes, sesquiterpenes, isoprenoids, alkaloids, and phenols) and their functions have been elucidated for their antibacterial, antifungal and antioxidant activities and chitosan an animal natural extract has manifested to be important in the management of plant diseases [4-7]. So, it is prerequisite to develop eco- friendly approaches and their wide practice for sustainable agriculture.

2. CHITOSAN (CS)
Chitosan is a structural linear polysaccharide present in exoskeletons or shells of crustaceans include shellfish, shrimp, crabs, lobsters and other microorganisms like green algae and cell wall of fungi [8]. It is the second most natural polysaccharide available after cellulose [9]. The main constituents with various compositions present in chitosan are D–glucosamine (deacetylated units) and N–acetyl–D–glucosamine (acetylated units) linked by β - 1,4 glycosidic bonds [10].

Commercially it is made by treating the chitin of shrimp and crustacean with an alkaline substance like NAOH and some enzymes. CS is a nontoxic biodegradable and biocompatible basic polysaccharides use in various fields include processing and package of food industry [11], cosmetics industries [12], biomedicinal fields [13], sustainable agriculture, shielding of environment from harzardous chemicals [14] and wastewater management.[15]. In 1983, chitosan was certified as a food/ feed additive by 'The council of Food and Drug Administration' of United States.

Antimicrobial properties of chitosan have been elucidated in many research studies [16-17]. Chitosan also elicits the immunity of plants against many microbes especially fungi and bacteria [18-21]. Chitosan on synergy with the bacterial cell surface membrane inhibits the growth of bacteria [22]. In few studies the growth of plants was enhanced [21,23-24].

3. ANTIMICROBIAL NATURE OF CHITOSAN

Chitosan is a versatile structural polysaccharide derived from chitin and it has multifaceted applications in various fields including medicine, agriculture, pharmacy etc. Its efficacious role on microbes majorly relays on its degree of deacetylation, molecular weight, and pH. Many research studies have revealed antimicrobial activity of chitosan against fungi, bacteria, and virus; however, specific mechanism of action is not yet fully understood. As of now, the following possible hypothesis mechanisms have tried to explicate chitosan antimicrobial activity.

3.1. CS enhances membrane permeability of microorganisms

In this hypothesis antimicrobial mechanism probably relays on the alteration of membrane permeability of microorganisms. Cell walls of Gram-positive bacteria is largely consisting of peptidoglycan with teichoic acid which gives negative charge to the cell surface (due to the presence of phosphate groups), whereas Gram-negative bacteria outer membrane of cell wall possess lipopolysacharides, which imparts strong surface negative charge. In acidic aqueous solutions, the NH_2 groups at C2 position of chitosan protonates to yield NH_3^+, which in turn forms electrostatic synergy with negatively charged groups (mostly phosphate groups of teichoic acids and lipopolysaccharides) located on the bacterial cell surfaces, leads to enhance the cell membrane permeability and subsequently perforation of intracellular constituents which leads to death of the cell [25]. In few *in vitro* research studies killing kinetics, cellular perforation measurements, estimation of membrane potential, electron microscopy and transcriptional factors response analysis have given a speculation that antimicrobial mechanism of low molecular weight (LMW) chitosan is due to electrostatic interactions of protonated amine groups with negatively charged teichoic acids of bacterial cell walls of Gram-positive bacteria *Staphylococcus aureus* [26]. In other research studies, Chitosan has shown more bactericidal effect in Gram +ve bacteria (*Lactobacillus plantarum, L. brevis, L.bulgaricus*, *Bacillus megaterium, B. cereus, Staphylococcus aureus*) than in Gram –ve bacteria (*E. coli, Pseduomonas fluorescens, Vibrio parahaemolyticus, Salmonella typhimurium*) [27]. Similarly, in a study, protonated chitosan has increased *E. coli* membrane permeability and that promoted lysis of membranes [28].

Similar mechanisms may also be proposed to fungal pathogens due to presence of phospholipids in their membrane. Protonated amine groups of chitosan, at low pH interacts with negatively charged phosphate groups of carbohydrate side chains of fungal cell wall proteins and decrease the negative charge that leads to alteration of important metabolic pathways [29].

In a study, antifungal activity against the pathogenic yeast *Candida albicans* was shown by LMW chitosan by decreasing the cell surface negative charge. Sialic acid, a constituent of cell wall glycoprotein of *C. albicans*, which provides negative charge on the cell wall surface [30-31].

3.2. CS interaction with nucleic acids –DNA/RNA

It supports that LMW, and nano size chitosan particles can be able to easily pass into cytoplasm of the microorganisms and interact to the negatively charged nucleic acids- DNA and RNA by electrostatic interactions and alter the downstream mechanisms such as transcription and translation [32-34].

3.3. Metal chelating nature of CS

The protonated chitosan chelates many of the divalent metal ions which are essential for microbial growth, enzymatic functions, membrane integrity and other [35-39]. Less availability of essential metal ions also one of the reasons for increase of microbe's cell wall permeability and sensitive to several chemicals or antibiotics [40].

3.4. Cell surface blocking nature of CS

As per this hypothesis, chitosan forms as a thick layer on the cell surface of the microbes and became a barrier for intake of essential nutrients, mineral and oxygen as well as excretion of their metabolic products, and hence it leads to death of the cells [41-42].

4. ANTIFUNGAL ACTIVITY OF CS

Chitosan is a broad-spectrum fungicide and shown fungicidal properties against many plant pathogen diseases [43-44]. It can suppress the growth and development of fungi at different stages of life processes. It showed great antifungal activity with the pathogen of rice sheath blight [45]. Growth of candida species are significantly inhibited at the low concentrations of chitosan hydrochlorides i.e., 0.0025% [46]. Radial and submerged growth of *Alternaria solani* was inhibited by chitosan at 1mg/ml and gave protection against blight pathogens to tomato plants. Control of fusarium wilts and grey mould was observed in soil amendment with chitosan [37]. Chitosan also conclusively supressed the growth and development of *Botrytis cinerea*, bunch rot, gray molds of the wine grape crops [47]. Research studies elucidated that N-benzyl chitosan a chitosan derivative at 1000 mg/L showed effective inhibition of spore germination in *Pythium debaryanum* and *Fusarium Oxysporum* [48].

5. ANTIVIRAL ACTIVITY OF CS

Chitosan acts as an anti- viral agent by controlling the viral infections in plants [49]. Studies revealed that chitosan associated antiviral activity was extremely limited when compared with anti-bacterial and antifungal activity [50]. Chitosan antiviral mechanism was assessed in animals, plants, and microorganisms [51-52]. Low molecular weight chitosan inhibits local necrotic lesions persuaded by *Tobacco mosaic virus* up to 90% [53]. In several images of electron microscope of TMV suspension of CS treated plants showed decreased number of virus particles [54]. In a study indicated that chitosan can promotes intracellular degradation of TMV by stimulating the hydrolases activity [55].

6. CHITOSAN IN CONTROL OF PLANT DISEASES

Plants have natural defense mechanism against pest and pathogens attacks. During pathogen attack plants make the alterations by developing hypersensitive response (HR) which leads to necrosis at the site of infection, reactive oxygen species (ROS) fabrication in uninfected parts of plants and stimulate expression of mechanical and chemical barriers. Further, stimulation of genes related to defense` mechanism and their enhanced expression leads to production of substances - phytoalexins, terpens, pathogenesis related proteins (PR). Other enzymes which are associated with defense mechanisms are phenylalanine ammonia lyase (PAL), polyphenol oxidases (PPOS) and peroxidases [ascorbate peroxidase (APX) and guaiacol peroxidase (G-POD)] [56-58].

Chitosan is considered as a stimulator or an elicitor for plant resistance because it interacts with cellular DNA in the plant and activate many biochemical mechanisms related to plant resistance. However, the exact mechanism for plant resistance by chitosan is still not well understood. The interaction of polycationic chitosan with negatively charged lipids on the cell wall surface might be the cause for the elicitor activity of chitosan. In turmeric plants foliar spray of chitosan has enhanced chitinase and chitosanase activity which has further shown resistance against rhizome rot disease caused by *Pythium aphanidermatum* [59]. In a field experiment, foliar application of chitosan has induced the activity levels of defense enzymes such as protease inhibitors (PI), β-1,3 glucanases, peroxidases (PO) and polyphenol oxidases (PPO) in the leaves and rhizomes of turmeric plants which further might involve in the formation of lignin to restrict the entry and movement of fungal pathogens in the plant system [60]. Many studies well documented that low molecular weight chitosan acts as a strong elicitor which is capable to induce defense mechanisms in plants [61-65]. Chitosan treatment on plants observed the production of many enzymes and molecules that are involved in defense mechanisms and development of mechanical and chemical barriers [56,66]. In few studies chitosan leads to programmed cell death due to the hypersensitive response around the site of infection [67]

CONCLUSION

Chitosan is a naturally available novel polysaccharide has many applications in various fields including in agriculture, medicine, and pharmaceuticals. It can be used as antimicrobial agent, anticancerous agent, plant growth promoter, scaffold for tissue engineering and many. In agriculture, it can be used for seed treatment and as plant resistance inducer and biopesticide. Its application is ecofriendly, inexpensive, farmer friendly and provides crop protection from various pests and pathogens.

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