

# A future perspective in crop protection: chitosan and its oligosaccharides

## Abstract

Chitosan and its oligosaccharides have received much interest for potential application in agriculture, biomedicine and biotechnology due to their biocompatibility, biodegradability and bioactivity. Synthetic bactericide treatment has been the main method for controlling diseases, there is a growing international concern over the indiscriminate use of synthetic compounds on crops because of the possible harmful effects on human health and the emergence of pathogen resistance to bactericides. Thus there is a worldwide trend to explore new alternatives in order to reduce the use of synthetic chemical agents. This review focuses on the biological activities of chitosan and its oligosaccharides based on the latest research results. Among the novel families of biological macromolecules, whose relevance is increased in research, are chitosan and chitoooligosaccharides (COS). Both become a promising alternative treatment due to its antimicrobial activity. They have a wide scope of application in the food, pharmaceutical, agricultural industries. COS are known to have eliciting activities leading to a variety of defence responses in plants in response to microbial infections to reduce the negative impact of diseases on yield and quality of crops. This review reiterates the properties and uses of chitosan and its oligosaccharides, and focuses on their applications on plant bacterial infections.

**Keywords:** chitosan, chitoooligosaccharides (COS), crop, antimicrobial activity

Volume 1 Issue 1 - 2014

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**Received:** May 06, 2014 | **Published:** May 17, 2014

## Introduction

Now-a-day's new approaches and practices are being developed for sustainable agriculture for various crops and vegetables. Therefore, various work is going on chitosan association for different plants growth in various laboratories of India. Chitosan and its oligosaccharides are natural molecule that are applied to crops with the aim of reducing or replacing more costly and environmentally damaging chemical bactericides. With reduced input costs and the potential for increased yields, farmers could gain substantial benefits from these applications of chitosan and its oligosaccharides to crops.

Chitosan is one of the most abundant natural amino polysaccharides extracted from the exoskeleton of crustaceans and insect, from fungal cell walls, etc. These substances have a wide variety of applications in agricultural and biotechnological industries.<sup>1,2</sup> Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, are chitin and its main derivative, chitosan. Both are the simplest linear polysaccharide composed of  $\alpha$ , 1-4 linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) with various compositions of these two monomers. Chitin and its derivatives have become a promising alternative treatment due to its natural character, antifungal activity and elicitation of defense responses in plant tissue. Therefore, recent studies on chitosan have attracted interest in converting it to more soluble COS, which possess a number of interesting biological activities, such as antibacterial and antifungal.<sup>3</sup> Chitosan and COS have attracted considerable interest due to their biological activities, namely, antimicrobial,<sup>4-11</sup> and enhanced calcium and ferrum absorption<sup>12-17</sup> and so on.

Studies on the biological activities of chitosan and its oligomers have been increasing, as no single type of chitosan or its oligomers exerts all of the above bioactivities. Moreover, different chitosan derivatives

and enzymatic products have different structures and physicochemical properties, which may result in novel bioactivities or novel findings in known bioactive compounds. Chitosan oligosaccharides also have been shown to induce various plant defense-related cellular responses and possess by themselves antimicrobial properties against a wide spectrum of phytopathogens.<sup>18,19</sup> The biological activity of COS is known to depend on their structure. Chitosan derivatives have a wide scope of application and regulate the immune system of plants and induce the excretion of resistant enzymes. Moreover, chitosan not only activates the cells, but also improves its disease and insect resistant ability. There is a great concern about the consequences of utilization of chemicals on health and environment, so other means of controlling diseases have become a vehement necessity. There are other well-known problems in agriculture sector, such as: disease resistance to bactericides, fungicides and high costs to develop new products, so the scientists are interested in biological control agents and naturally occurring fungicides. Keeping in view the important role of chitosan and COS in agriculture, this review is aimed at investigating the efficacy of chitosan and its derivative on crop protection.

## Chitosan

Chitosan is derived from chitin, a polysaccharide found in exoskeleton of shellfish such as shrimp, lobster or crabs and cell wall of fungi.<sup>20</sup> Chitosan poly (1,4)-2-amino-2-deoxy- $\beta$ -D glucose, is a deacetylation product of chitin, a polysaccharide second by the prevalence in nature after cellulose.<sup>21</sup> Chitin is an abundant natural polysaccharide produced by arthropods and crustaceans. It is found in the wide number of invertebrates (crustacean's exoskeleton, insect's cuticles) and it is also an ingredient of cell walls in fungi yeast and algae and as a structural polysaccharide in basidiomycetes and filamentous fungal cell walls, constituting almost 16% of

the dry weight of the organism.<sup>22</sup> Chitosan is the simplest linear polysaccharide composed of  $\alpha$ , 1–4 linked D–glucosamine (GlcN) and N–acetyl–D–glucosamine (GlcNAc) with various compositions of these two monomers.<sup>1</sup> Chitosan are highly basic polysaccharides with unique properties like the ability to form films<sup>23</sup> to react with polyanions<sup>24</sup> as well as to chelate and remove metal ions.<sup>25</sup> The positive charge of chitosan confers to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as cosmetology (lotions, hair additives, facial and body creams),<sup>26</sup> food (coating, preservative, antioxidant, antimicrobial),<sup>27–32</sup> biotechnology (chelator, emulsifier, flocculent)<sup>9,33</sup> pharmacology and medicine (fibers, fabrics, drugs, membranes, artificial organs)<sup>34–36</sup> and agriculture (soil modifier, films, fungicide, elicitor).<sup>37–40</sup> The molecular weight has important effect on the biological activity and absorption of chitosans *in vivo*.<sup>41–43</sup> Chitosan and its derivatives have shown various functional properties and made them possible to be used in many fields including, food,<sup>44</sup> cosmetics<sup>2</sup> biomedicine,<sup>45</sup> agriculture,<sup>46</sup> environmental protection<sup>47</sup> and wastewater management.<sup>48</sup> Further, the biodegradable,<sup>49</sup> non–toxic<sup>50</sup> and non–allergenic features of chitosan especially encourage its potential use as a bioactive material.<sup>51</sup>

The Food and Drug Administration (FDA) agency in U.S. approved chitosan as a feed additive in 1983. Chitosan has been widely applied in functional food environmental protection and biotechnology.<sup>52,53</sup> Chitosan has been reported to have antifungal and antimicrobial effects.<sup>54,55</sup> Chitosan is a static or cidal agent of microbial growth, useful in agriculture, household goods manufacturing<sup>4</sup> and the applications and mode of action have been reviewed.<sup>56</sup> Stimulation of plants immunity against microorganisms (bacteria and fungi).<sup>57–61</sup> It suppresses the growth of bacteria probably because of binding to the cell surface.<sup>9</sup> Recently, some researchers reported that chitosan enhanced plant growth and development.<sup>60,62,63</sup> The molecular weight has important effect on the biological activity and absorption of chitosan *in vivo*.<sup>41,42</sup> Lee et al.,<sup>64</sup> reported that chitosan reduced the size of stomatal aperture and inhibited light induced stomatal opening by inducing reactive oxygen species (ROS) including super oxide and hydrogen peroxide which inhibit stomatal opening and promote stomatal closing.

## Source

Chitin and chitosan are found as supporting materials in many aquatic organisms (shells of shrimps, crabs, bone plates of squids and cuttlefishes), in many insects (mosquitoes, cockroaches, honey bees, silkworms, *Drosophila melanogaster*, *Extatosoma tiaratum* and *Sipyloidea sipyilus*), in terrestrial crustaceans (*Armadillidium vulgare*, *Porcellio scaber*), in nematode, in mushrooms (*Agaricus bisporus*, *Auricularia auriculajudae*, *Lentinula edodes*, *Trametes versicolor*, *Armillaria mellea*, *Pleurotus ostreatus*, *Pleurotus sajo-caju* and *Pleurotus eryngii*) and in some microorganisms (yeast, fungus and algae diatom).<sup>65–69</sup>

## Chitosan application on crops

Chitosan effects on plant response were first characterized as an elicitor. It was shown to be able to activate plant defensive genes through the octadecanoid pathway.<sup>70</sup> According to the defensive gene induction activity, chitosan was proved to induce disease resistance in several plants, with pathogen and plant cultivar specificity.<sup>71,72</sup> Chitosan was also involved in the stomatal response where stomatal opening provides access to inner leaf tissue for plant pathogens,

so narrowing stomatal apertures may be advantageous for plant defense. The stomatal aperture of tomato and *Commelina communis* was reduced when the epidermis was treated with chitosan.<sup>64</sup> It was found in pepper plant that foliar application of chitosan decreased transpiration and reduced water use by 26–43%, while maintained biomass production and yield. Hence, chitosan might be an effective antitranspirant to conserve water use in agriculture.<sup>73</sup> Hirano et al.,<sup>74</sup> reported that coating of seeds with depolymerized chitosan or its oligosaccharides typically increased the chitinase activity in seedlings by 30–50%, unless the seeds had a hard cuticle. Low molecular weight of chitosan (5kDa) induced the accumulation of phytoalexins in plant tissue, decreased the total content and changed the composition of free sterols producing adverse effects on infesters, activated chitinase, beta–glucanase, lipoxigenases and stimulating the generation of reactive oxygen species.<sup>75</sup>

Application of chitin and chitosan to soybean leaf tissues increased activities of Phenylalanine Ammonia Lyase (PAL) and Tyrosine Ammonia–Lyase (TAL). The elevation of PAL and TAL activity was dependent on the chain length of the oligomers and time after treatment.<sup>76</sup> Xianling et al.,<sup>77</sup> indicated that seeds of mulberry cultivar were coated with chitosan solution at 3% prepared from silkworm chrysalises increased the respiration rate of germination seeds, root vigor, chlorophyll, protein content and peroxidase in seedlings as well as nitrate reductase and amylase activities. Nicholson et al.,<sup>78</sup> reported that increases in PAL activity have been demonstrated to be one of the earliest responses of plants to the onset of stress by pathogen infection and are considered as an indication of resistance. Since PAL is the key enzyme in the phenylpropanoid pathway, its activity leads to synthesis of phenols, which are compounds associated with expression of resistance.

Yue et al.,<sup>79</sup> studied maize seeds and reported that chitosan concentration at 2–4g/litre resulted in a positive effect on endogenous hormone content, alpha–amylase activity and chlorophyll content in seedling leaves. Khan et al.,<sup>62</sup> found that on the first day after foliar application, chitosan pentamer decreased the net photosynthetic rate of maize and subsequently there was an increase on day 3 of 10–18% over the control. This increase was related with the increases in stomatal conductance and transpiration rate, while the intercellular CO<sub>2</sub> concentration was not different from the control plants. Chitosan also enhanced the Rice Endosperm Kinase (REK) in RNA expression, which was completely abolished at either 12 or 24h by cycloheximide. However, high/low temperature and the environmental pollutants, ozone and sulfur dioxide, failed to enhance the REK mRNA expression, as determined using *in vivo* system. These results strongly indicated a function for REK in defense/stress responses in rice photosynthetic tissues.<sup>80</sup> Ohta et al.,<sup>81</sup> reported that chitosan application to the soil at sowing time remarkably enhanced plant growth and flowered 15 days earlier than the control. Moreover, a greater number and weight of flowers were produced by chitosan application. Rapeseed (*Brassica chinensis*) coated with small molecular weight chitosan showed positive effects on germination index, growth of seedlings and root length.<sup>82</sup> Chibu and Shibayama<sup>83</sup> studied chitosan application on early growth of four crops: soybean, lettuce, tomato and rice. The results showed that chitosan at 0.1 or 0.5% increased leaf area, leaf dry weight and leaf length of soybean, lettuce and rice whereas chitosan at 0.1% showed positive effects on leaf area, leaf length and dry weight of tomato. Seeds of non– heading chinese cabbage cv. Dwarf hybrid No.1 dressed with chitosan at the rate 0.4–0.6mg/g of seeds and leaf spraying with 20–40 microgram/ml increased total fresh weight, leaf

area, plant height, root length, soluble protein and soluble sucrose in leaves, while the content of crude fiber decreased.<sup>84</sup>

Utsunomiya et al.<sup>85</sup> studied the effect of using chitosan oligosaccharides as a soil conditioner with high and low nitrogen on the flowering and fruit growth of purple passion fruit. They found that the numbers of flowers, harvested fruits, fruit weight and juice production were increased significantly by the soil conditioner under high nitrogen. Under conditions of low nitrogen, the soil conditioner reduced flower formation in the first growing season but had little effect on flowering in the next season. This treatment increased fruit and juice weights to nearly the same degree as those observed in the high nitrogen without soil conditioner.

Harada et al.<sup>86</sup> reported that application of chitosan in the field increased shoot growth, branch length, node number per plant and seed yield of soybean and total root length per plant increased by chitosan application in the pot experiment. Krivtsov et al.<sup>87</sup> found that wheat seed soaked with aqueous solution of polymer (10–20kDa) or oligomer (2–4kDa) at concentration of 50mg/litre for 3 to 18 hours increased stem and root lengths of seedling by six days after seed treatment. Foliar application at the panicle initiation stage with 0.01 or 0.02% solution of oligochitosan two times (five days apart) increased spike weight, 1,000-grain weight and grain yield by 16%. Ali et al.<sup>88</sup> revealed that the production of nodule fresh weight and nitrogen fixation of soybeans decreased at early growth stage (28 days after sowing) in soil supplemented with chitosan 0.1–0.25%, but increased at the later growth stage. Chibu and Shibayama<sup>83</sup> reported that the top dry weight, leaf area, number of first order thick lateral and second order lateral roots of radish seedlings were increased by chitosan application at 0.1%. Seed of soybeans soaked with chitosan solution at 1000ppm for six hours and cultured at 25 degree C for 6 days increased germination percentage, hypocotyl thickness, total length and fresh weight of sprouts by 4, 5, 2 and 1% respectively.<sup>64</sup>

Chitosan has been shown to trigger defense mechanisms in plants. Plants treated with chitin and chitosan produce chitinase that breaks down the chain of chitin and chitosan into more soluble form. Loschke et al.<sup>89</sup> reported that chitosan induces the expression of a variety of genes involved in plant defense response that, in some cases, result in increased synthesis of secondary plant metabolites. Chitosan influences pathways involving jasmonic acid.<sup>70,90,91</sup> Jasmonate exhibits some activities similar to the plant hormone Abscisic Acid (ABA), which plays a key role in the regulation of water use by plants.<sup>92</sup> Increased levels of ABA result in closure of the plant's stomata and reduced transpiration.<sup>93,94</sup> Thus, manipulating the ABA signaling pathway offers the possibility to reduce water consumption by plants.<sup>95</sup> Jwa et al.<sup>96</sup> reported that as a fungal elicitor, chitosan enhanced the Rice Endosperm Kinase (REK) mRNA expression, which responds to the critical signals mediating plant self defense/stress response, namely Jasmonic Acid (JA), Salicylic Acid (SA) and hydrogen peroxide. Klusener et al.<sup>97</sup> revealed that yeast (*Saccharomyces cerevisiae*) elicitor and chitosan, both elicitors of plant defense response, activated this current activation requiring cytosolic NADPH and induced elevations in the concentration of free cytosolic calcium and stomatal closure in guard cells. Wheat and barley damaged at 2.5µg/ml of vanadium were recovered by treatment with 10–100µg/ml chitosan.<sup>98</sup> Lee et al.<sup>64</sup> reported that chitosan, a component of fungal cell walls, reduced the size of stomatal aperture and inhibited light induced stomatal opening in tomato epidermis by inducing Reactive Oxygen Species (ROS) such as superoxide and H<sub>2</sub>O<sub>2</sub>, which inhibit stomatal opening and promote stomatal closing.

## Chit oligosaccharides

Chito oligosaccharides (COS) has attracted considerable interest due to their biological activities, namely, antimicrobial.<sup>5–11</sup> Studies on the biological activities of chitosan and its oligosaccharides have been increasing, as no single type of chitosan or its oligomers exerts all of the above bioactivities. Moreover, different chitosan derivatives and enzymatic products have different structures and physicochemical properties, which may result in novel bioactivities or novel findings in known bioactive compounds. Due to its cationic character, chitosan presents a wide variety of physicochemical and biological properties, including antimicrobial, antioxidant and antihypertensive properties.<sup>99</sup> These properties make it suitable for numerous applications in different fields, for instance, medicine (pharmacology, cosmetics), biotechnology, agriculture, the food industry (nutritional enhancement and food processing), the environment (waste and water treatment) and the textile industry.<sup>99,100</sup> In addition, its lack of toxicity and allergenicity, as well as its biocompatibility, biodegradability and bioactivity makes it a very attractive substance for use in biomaterial design. However, its poor solubility in solutions of neutral and basic pH hinders its use in many industrial applications. Therefore, there is considerable interest in improving chitosan solubility and different strategies have been developed for instance, the partial hydrolysis of chitosan to obtain Low Molecular Weight Chitosan (LMWC) and COS, which are soluble in water. COS are defined as chitosan with a Degree of Polymerization (DP) less than 20 and MW of up to 3900Da.<sup>3, 54,101,102</sup>

Hydrolysis of chitosan can be carried out by chemical, physical and enzymatic methods. Chemical hydrolysis is performed at high temperatures under highly acidic conditions, mainly using HCl or HNO<sub>2</sub> and produces a large amount of glucosamine.<sup>103</sup> Due to the complexity of controlling the progress of the reaction, these treatments also result in the formation of secondary compounds that are difficult to remove. By physical methods such as irradiation with low-frequency ultrasound (20kHz), partial depolymerization is obtained, reducing the average MW from 2000kDa down to 450kDa or from 300kDa to 50kDa; however, the reduction of MW is limited.<sup>102</sup> Enzymatic methods, such as the use of chitosanases and nonspecific enzymes, seem to be generally preferable to chemical methods because the reaction is performed under more gentle conditions and the MW distribution of the product is more controllable. The expensive cost of chitosanases limits their wide application on an industrial scale, even using immobilized enzymes.<sup>104</sup> On the other hand, nonspecific enzymes are inexpensive and commercially available and have been used in the industry for years to produce COS with relatively low cost. Lin et al.<sup>105</sup> reported the hydrolytic susceptibility of chitosan to a wide range of enzymes, including glycanases, proteases and lipases derived from bacterial, fungal, mammalian and plant sources. Enzymatic chitosan hydrolysis can be performed either with soluble or immobilized enzymes column or membrane reactors. Certain chitosan oligomers have physiological functions including the induction of phytoalexins.<sup>106</sup>

## Antimicrobial activity

The antimicrobial activity of chitosan and its oligosaccharide derivative has been recognized and is considered to be one of the most important properties, corresponding directly to their possible biological applications.<sup>10,107</sup> Allan et al.<sup>4</sup> first reported chitosan and its derivatives had broad-spectrum antimicrobial effects. Since then many studies have been performed on the antimicrobial activity of



chitosan and its derivatives and oligosaccharides, confirming that chitosan showed antimicrobial properties with bacteria, yeasts and fungi. The antibacterial activities of six chitosans and six chitosan oligomers with different molecular weights (Mw) were examined against four gram-negative (*Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium* and *Vibrio parahaemolyticus*) and seven gram-positive bacteria (*Listeria monocytogenes*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Lactobacillus brevis* and *Lactobacillus bulgaricus*) by No et al.<sup>6</sup> They found that chitosan showed higher antibacterial activities than chitosan oligomers and markedly inhibited the growth of most of the tested bacteria, although the inhibitory effects differed with the Mw of chitosan and the bacterial species. Chitosan generally showed stronger bactericidal effects on gram positive bacteria than gram-negative bacteria at a concentration of 0.1%. The minimum inhibitory concentration (MIC) of chitosan ranged from 0.05% to more than 0.1% depending on the bacterial species and the Mw of the chitosan. As a chitosan solvent, 1% acetic acid was effective in inhibiting the growth of most tested bacteria except for *Lactobacillus*, which was more effectively suppressed with 1% lactic or formic acids. The antibacterial activity of chitosan ranged from 0.05% to more than 0.1% depending on the bacterial species and the Mw of the chitosan. As a chitosan solvent, 1% acetic acid was effective in inhibiting the growth of most tested bacteria except for *Lactobacillus*, which was more effectively suppressed with 1% lactic or formic acids.

The antibacterial activity of chitosan was inversely affected by pH and exerted better effects at a lower pH value. Uchida et al.,<sup>9</sup> previously reported that the MIC of chitosan for *E. coli* and *S. aureus* were 0.025% and 0.05%, respectively. Hence, chitosan was recognized as the best candidate among natural antimicrobial preservatives, although the antimicrobial activity and MIC acquired from different researchers differs, probably due to differences in the experimental methods, type of chitosan or pH. However, Zheng et al.,<sup>108</sup> used *E. coli* and *S. aureus* to study the antimicrobial activity of chitosan with different molecular weights (Mw). They found that chitosan with Mw below 300kDa, the antimicrobial effect on *S. aureus* was strengthened as the molecular weight increased; in contrast, the effect on *E. coli* was weakened. The antibacterial activities of water-soluble N-alkylated disaccharide chitosan derivatives against *E. coli* and *S. aureus* were also investigated by Yang et al.<sup>109</sup> They found that the antibacterial activity of chitosan derivatives was affected by the degree of Disaccharide Substitution (DS) and the kind of disaccharide present in the molecule. Regardless of the kind of disaccharide linked to the chitosan molecule, a DS of 30–40%, in general, exhibited the most pronounced antibacterial activity against both test organisms. *E. coli* and *S. aureus* were the most susceptible to cellobiose chitosan derivatives and maltose–chitosan derivatives, both with DS values of 30–40%, among the various examined chitosan derivatives. Although the disaccharide chitosan derivatives showed less antibacterial activity than native chitosan at pH 6.0, they exhibited higher activity at pH 7.0. The antibacterial activity of the chitosan derivatives (DS 30–40%) against *E. coli* increased as the pH increased above 5.0 and reached a maximum around pH 7.0–7.5. The effect of pH on the antibacterial activity of chitosan derivatives against *S. aureus* was not as significant as that observed with *E. coli*. Population reductions of *E. coli* or *S. aureus* in nutrient broth increased markedly when the concentration of chitosan derivatives was increased from 0 to 500mg/kg, while no marked increase in population reduction was found with further increases, even up to 2,000mg/kg. Additionally, chitosan has shown

inhibitory effects on the growth of fungi and other microorganisms, especially plant pathogens. Chitosan can induce plants to produce defence enzymes (chitosanases) with antimicrobial activity. It has a higher antimicrobial activity than chitin because it carries a positive charge.

Hirano et al.,<sup>33</sup> studied the relative molecular weights of chitosan on the inhibition of plant pathogens. The results indicated that COS (DP2–8) and partially degraded Low Molecular Weight Chitosan (LMWC) showed higher inhibitory activities on *Fusarium oxysporum*, *Phomopsis fukushi* and *Alternaria alternata* than high-molecular-weight chitosan. Uchida et al.<sup>9</sup> found that the inhibition of fungi and bacteria by COS with higher Degrees of Polymerization (DP) was much stronger than those by chitosan and COS with lower DP; simultaneously, their inhibitory effects increased with increasing DD. This result was confirmed by later research.<sup>110</sup> Jeon et al.,<sup>14</sup> produced and isolated three kinds of COS using an ultra filtration membrane bioreactor; among these, the COS with Mw of 5,000–10,000 showed strong antimicrobial activity on the tested pathogens. Later, they produced a COS with a DP of 3–6 by the same methods, which showed a higher inhibitory effect on *E. coli* with increasing concentration; a 0.5% COS solution completely inhibited the growth of *E. coli*. In our previous study, we also studied the antimicrobial activity of partially hydrolyzed COS against the common bacteria, molds and yeasts in found food and found it to be much higher than that of chitosan, with an MIC of 1–10g/L and the inhibitory effects were enhanced with increasing COS concentration. Moreover, the relations between their structures and antimicrobial effects were also examined. It was found that the antimicrobial activity of COS was correlated with the content of protonated amino groups and relative molecular weights. Additionally, when COS was applied as a preservative in apple juice, the storage period of juice at 37°C was prolonged from nine days to 70days with a COS concentration of 4g/L, showing good preservative effects.<sup>10</sup> Chitin, chitosan and their oligomers have been reported to exhibit elicitor activities in several plants and have been widely used as elicitors for the induction of secondary products in plant cell cultures. When attacked by pathogens such as fungi, bacteria and viruses, higher plants have various defense reactions including the production of phytoalexins, enzymes such as chitinase and  $\beta$  glucanase, proteinase inhibitors, hydroxyproline-rich glycoproteins, proteinase, active oxygen species as well as lignification. Chitin oligomers were active as elicitors of defense reactions in higher plants, whereas chitosan oligomers had almost no eliciting activity. However, higher chitosan oligomers, e. g., octamers, were efficient elicitors for inducing pisatin accumulation and inhibiting fungal growth. These results suggest that elicitor activities of chitosan oligomers are highly dependent on their polymerization and the presence of N-acetyl glucosamine. From the above, we can conclude that although there are many reports discussing chitosan's antimicrobial activity in different conditions with conflicting results, they all confirmed that chitosan and its oligosaccharides have strong antimicrobial effects and are safe for human use. Hence, the antimicrobial characteristics chitosan and its oligosaccharides present a profitable potential for developing natural food preservatives for food-processing applications and functional-food additives.

## Conclusion

After various recent research findings, chitosan has applications in numerous fields, as described in many review articles. This naturally occurring molecule with interesting antimicrobial and

eliciting properties has been getting more attention in recent years. This molecule can be used in a number of ways to reduce plant disease levels and prevent the development and spread of diseases, thus preserving crop yield and quality. The potent effect of chitosan on plant diseases control is from its antimicrobial properties and plant innate immunity elicited activity. The antimicrobial activity is influenced by several factors, such as MW, DDA, solubility, positive charge density, chemical modification, pH, concentration, chelating capacity and type of micro-organism. Chitosan has also become a postharvest promising treatment for fruits due to its natural character, antimicrobial activity and elicitation of defence responses. In spite of the chitosan advantages, the poor solubility of chitosan is the major limiting factors in its utilization. Therefore, several researchers have started to modify a chitosan molecule to produce high-antimicrobial active derivatives COS. Our review suggests that chitosan and COS can be used as potent antibacterial molecule in biological systems.

### Future perspectives

Our review revealed that chitosan and COS have a potential to develop an alternative bactericide to prevent plant infections. Interesting theoretical and applied findings were gathered in recent years, whereas more are needed to examine the mechanisms governing the mode of action of these compounds. In the case of antimicrobial mode of action, future work should aim at clarifying the molecular details of the underlying mechanisms and their relevance to the antimicrobial activity of chitosan. In addition, participation and collaboration of research institutes, industry and government regulatory agencies will be the key for the success of the antimicrobial mechanism when applied in large scales. Therefore, future research should be directed towards understanding their molecular level details, which may provide insights into the unknown biochemical functions of chitosan and COS as well as help to accelerate their future and might assist in the goal of sustainable agriculture.

### Acknowledgements

The Authors wish to thank University Grant Commission (UGC, Government of India) New Delhi for financial support in form of Post Doc Fellowship (F.15-1/2012-13/PDFWM-2012-13-OB-UTT-17432). We are thankful to Department of Plant physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, as well as Prof. A. Hemantaranjan for providing necessities of this work.

### Conflict of interest

The author declares no conflict of interest.

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