Agricultural Reviews, 39(2) 2018 : 163-168
Print ISSN:0253-1496 / Online ISSN:0976-0539
AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com

Chitosan: An elicitor and antimicrobial Bio-resource in plant protection

Akansha Singh*[1], Kalpana Gairola[1], Vinod Upadhyay[2] and J. Kumar[1]

Department of Plant Pathology,
G.B. Pant University of Agriculture and Technology Pantnagar-263 145, Uttarakhand, India.
Received: 08-05-2017 Accepted: 08-02-2018 DOI: 10.18805/ag.R-1723

ABSTRACT

Pesticide resistance and environment threat due to injudicious use of chemical pesticides for disease management employs the alteration in management practices. Chitosan, a deacetylated chitin derivative, behaves like a general elicitor, inducing a non-host resistance, and prime the plants for systemic acquired resistance in addition to this Chitosan has high antimicrobial activity against a wide range of pathogenic and spoilage microorganisms, including fungi and bacteria. The use of chitosan in agriculture and in food systems should be based on sufficient knowledge of the complex mechanisms of its elicitor and antimicrobial mode of action. In this article we a number of studies on the investigation of chitosan antimicrobial and resistance inducing properties and application of them in agriculture sector have been summarized.

Key words: Antimicrobial, Chitosan, Elicitor, Defense mechanism, Induced resistance.

Chitosan, is a biopolymer derived by partially deacetylation of chitin (more than 75% degree of deacetylation), mostly from the exoskeletons of crustaceans (such as shrimp, crab, lobster etc.) and insects. Recent advances in fermentation technology suggest that the cultivation of fungi can provide an alternative source of chitosan. Cell wall of *Aspergillus niger* and *Mucor rouxii* contain 45% and cell wall of *Penicillium notatum* contains 20% chitin (Arcidiacono and Kalpan 1992). Manipulation of fungal culture media and fermentation condition may provide chitosan of more consistent physico-chemical properties compared to that derived by deacetylation of crustacean chitin (Chatterjee *et al.,* 2005). For example, Chatterjee *et al.* (2009) isolated chitosan from *Mucor rouxii* cultured in molasses salt medium supplemented with plant growth hormones.

Structurally, chitin and chitosan are biopolymers composed of *N*-acetylated glucosamine and glucosamine units linked by â (1–4) glycosidic bonds. Both polymers have been shown to have various promising antimicrobial activities. It behaves like an elicitor, inducing a non-host resistance leading to systemic acquired immunity apart from this it also have antimicrobial activity. Its antimicrobial and elicitor properties are depends on its biological origin, degree of deacetylation, molecular weight, pH of the medium, concentration in solution and of course on type of pathogen.

Presently chitosan has paying more attention in agriculture sector because of its unique biological activities such as biodegradability, biocompatibility, non toxicity, viscosity, anti-microbial, resistance inducer, bio-film forming and food preservative capabilities (Hsu *et al.,* 2011, Hafdani and Sadeghinia 2011).

The present review will summarize antimicrobial and resistance elicitation properties of chitosan and a number of new efforts on usage of chitosan in plant protection.

Chitosan deivitization: Three types of reactive functional groups are present in chitosan: An amino group at the C-2 position of each deacetylated unit, as well as primary and secondary hydroxyl groups at the C-6 and C-3 positions, respectively, of each repeat unit. These reactive groups are readily subjected to chemical derivatization for improving compatibility and mechanical and physico-chemical properties of chitosan (Raafat and Sahl 2009). Recently derivatized chitosans such as salicyloyl chitosan (He *et al.*, 2011) Ofumaryl-chitosan (Feng *et al.*, 2011) arginine-functionalized chitosan (Tang *et al.*, 2010) and guanidinylated chitosan derivatives (Hu *et al.*, 2007) have been reported to have more ability to inhibition of bacterial growth and higher solubility than underivatized chitosan.

Methylatin in main chain of chitosan make it water soluble and enhance the chitosan antimicrobial activity and applicability in a large pH range (Goy *et al.*, 2009). Runarsson *et al.* (2007) synthesized series of methylated chitosaccharide derivatives that had antibacterial effects at pH 7.2, whereas they did not contribute to the antibacterial activity under acidic conditions. Yang *et al.* (2005) showed that *E. coli* and *S. aureus* were most susceptible to cellobiose chitosan derivative Degree of Substitution 30–40% and maltose chitosan derivative DS 30–40%, respectively. Antibacterial activity of the chitosan derivatives (30–40%)

*Corresponding author's e-mail: singh.akansha03@gmail.com
[1]Department of Plant Pathology, G.B. Pant University of Agriculture and Technology Pantnagar-263 145, Uttarakhand, India.
[3]Regional Agricultural Research Station, Assam Agricultural University, Gassaingaon-783 360, Assam, India.

against *E. coli* increased as the pH increased from 5.0 to pH of 7.0–7.5. N-alkylated disaccharide chitosan derivatives showed a higher antibacterial activity than the native chitosan at pH 7.0. Li *et al.* (2011) reported the antimicrobial activity of the xylan"chitosan conjugate was higher than native chitosan. Liu *et al.* (2000) prepared a kind of derivatized chitosan, N,O-carboxymethylated chitosan, that had higher antibacterial activity against *E. coli* than underivatized chitosan. Vallapa *et al.* (2011) using a tow step process (reductive alkylation and methylation) introduced quaternary ammonium groups to the chitosan surface and could significantly improve the antibacterial activity of the chitosan, especially in a neutral environment.

Broad spectrum defense responses elicited by chitosan include rising of cytosolic H^+ and $Ca^{2+,}$ activation of MAP-kinases, apposition, oxidative burst, hypersensitive response (HR), synthesis of abscissic acid, phytoalexins and pathogenesis related (PR) proteins. Wide spectrum antimicrobial activities of chitosan includes inhibition of mycelia growth, sporulation, spore germination, germ tube elongation via electrostatic interaction, membrane damage, Chitosan DNA/RNA interaction, metal ion chelation and deposition on microbe surfaces (Xing *et al.*, 2015).

RESISTANCE INDUCING MECHANISM OF CHITOSAN

Apart from antimicrobial activities chitosan is also a potential elicitor. It shows synergestic effect with other biocontrol agents such as *Trichoderma* species. Chitosan induces systemic acquired resistance by following mechanism:

i). Defense-related enzymes: Chitosan can induces resistance on yhe host by in creasingthe activities of several defense enzymes such as Phenyl alanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), catalases, super oxide dismutase, NADPH oxidase.

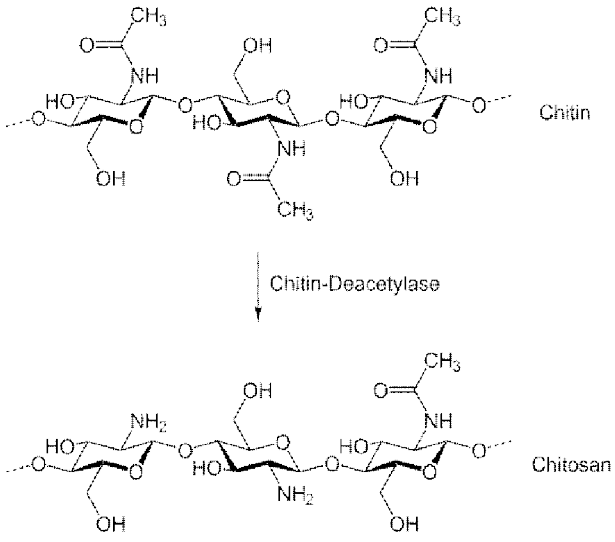
Phenylalanine ammonia-lyase (PAL) is an enzyme that catalyzes the L-phenylalanine to ammonia and trans-cinnamic acid. PAL is a key enzyme and committed for phenyl propanoid pathway, phenylalanine ammonia-lyase is induced in host tissues after pathogen infection of plant tissues and by abiotic elicitor treatments, such as chitosan. Cinnamic acid is the precursor of various compounds which are produced in phenyl propanoid pathway. Peroxidase is an oxidizing enzyme which oxidisase as phenol into quinine and also produces H_2O_2. NaOH oxidases catalyze the reaction of reactive oxygen species which is responsible for hypersensitive reaction and ultimately isolate the plant pathogen from normal healthy tissues.

ii) Histological defence induction

Chitosan was known to have eliciting activities leading to callose formation in host plants in response to microbial infections. The elicited callose apposition in plant tissues exerted a determinant role in limiting microbial spread in the early phase of pathogen infection there is increase in cytoplasmic streaming and accumulation of cytoplasm in the affected cell. Cytoplasmic aggregates contains cellular apparatus needed for cell wall strengthening which include: 1) formation of papillae (a deposit of callose, silicon, lignin and proteins) beneath the point of penetration ii) formation of lignotubers, lignified callose depositions that ensheath the invading hyphal tips and iii) cross linking of hydroxyproline rich glycoproteins, structural proteins of the cell wall also reinforces cell wall components.

iii). Secondary metabolites

a). Phenolic compounds: Chitosan produced elevated phenylalanine ammonia-lyase activity in plant, the levels of total phenolic content may also increase following chitosan treatments. As the major phenolic compound in has been reported to have various bioactive properties such as anti-oxidant, anti-microbial and anti-inflammatory activities.



Chitin and chitosan are Heteropolysaccharide. They are biopolymer of 2 acetyl amino-2-deoxy D glucopyranose bounded by β-1, 4-glycosidic bond. In contrast to chitin, chitosan amino groups are mostly deacetylated.

Table 1: Antimicrobial and defense elicitor mechanism of chitosan.

Defence mechanism elicited by Chitosan	Antimicrobial mechanism of Chitosan
Calcium transient	Membrane damage mechanism
Plasma membrane H+-ATPase inhibition	Chitosan-DNA/RNA interactions
Callose apposition	Metal chelation capacity of chitosan
Reactive oxygen species	Deposition onto the microbial surface
Hypersensitive response	Electrostatic interaction
Phytoalexins	
Systemic acquired resistance	
Pathogenesis related proteins	

Chlorogenic acid, caffeic acid, ferulic acid, umbelliferon, coumarin, phloretin are most common antimicrobial phenolic compounds and their concentration is high in chitosan primed cell.

The most complex poly phenols are lignins, tannin, phenylglycosides, flavonoids, anthocyanin and anthoxanthins. Lignification and suberisation renders the cell wall more resistant to mechanical pressure during penetration by fungal appressoria as well as more water repellant hus, it forms a barrier offering protection against microbial and chemical degradation. In the plant-pathogen interaction, the lignification of infected plant cell walls is a mechanism for disease resistance and provides plants with effective protection against pathogens.

b) Phytoalexin: Phytoalexins are low molecular weight antifungal and antioxidative compounds synthesized by plants in response to a pathogen challenge or induced by treatment with elicitors such as chitosan. In a narrow sense, phytoalexins tend to fall into several classes including terpenoids, isoflavonoid, alkaloids and phenolics. Chitosan can be extensively used for inducing phytoalexin accumulation in plant tissue and enhancing secondary metabolite yields (Eilenberg *et al*., 2010). Pisatin, rishitin, resveratrol, ipomeameron, coumarin, lubimin, phytotuberol are important phytoalexins. The application of chitosan as elicitors is a promising prospect in the production of biologically active phytoalexins and other secondary metabolites.

iv).Hypersensitive response: Rapid localized cell and tissue death around the site of infection is called as Hypersensitive reaction. The pathogen remained confined to necrotic lesions near the site of infection and killed due to starvation. Cytoplasmic condensation, DNA fragmentation, vacuolization of cytoplasm is the mechanism for HR. The cells surrounding the necrotic lesions are highly reactive and initiate the synthesis of antimicrobial compounds (phytoalexins) and signal molecules (salicylic acid).

v). Pathogenesis related protein: Plant proteins that are induced in pathological or related situations and give the resistance response are called as Pathogenesis related protein (PR proteins). These proteins are accumulated maximally 7-10 days after infection and indicate the attainment of systemic acquired resistance in by the plants. PR-2 (β-1,3glucanases), PR-3, PR-4, PR-8, PR-11 (Chitinases), PR-5 (Thaumatin like), PR-6 (Proteinase inhibitors), PR-9 (Peroxidases), PR-10 (Ribonuclease activity), PR-14 (Lipid transfer protein) etc. are important PR proteins and their expression are enhanced in chitosan treated cell (Hadwiger 2013).

B. ANTIMICROBIAL ACTIVITIES OF CHITOSAN

Chitosan is promising antimicrobial compound having antifungal antibacterial and antiviral properties. Antimicrobial action of chitosan is embraced by following mechanism:

i). Electrostatic interaction: Chitosan is partially deacetylated biopolymer number of reactive amino groups ($-NH_2$) are available which can be protonated. It is insoluble in water but soluble in dilute acidic solvents such as acetic acid and HCl in which amine group ($-NH_2$) in glucosamine units is converted into soluble protonated form $-NH_3^+$ (Silva

Table 2: Defence mechanism induced by chitosan against various pathogens.

Crop/ Plant	Pathogen	Disease	Defence mechanism	References
Rice	*Rhizoctonia solani*	Sheath blight	Increased Phenyl ammonia lyase, peroxidase, poly phenol oxidase	Liu *et al*., 2012
Cotton	*F. oxysporum f.sp. vasinfectum*	Vascular wilt	Phytoallexin accumulation	Awadalla and Mahmoud 2005
Potato	*Phytophthora infestans*	Late blight	Defence enzymes	Sajeesh, 2015
Potato	*Alternaria solani*	Early blight	PR Proteins	Kareem *et al*., 2014
Grapevine	*Uncinula necator*	Powdery midew	Polyphenol content and radical scavenging activity	Iriti *et al*., 2011
Citrus	*Penicilliumdigitatum*	Grey mold	Chitinase and Beta 1,3-glucanase	Guilli *et al*., 2016
Mustard	*Alternaria brassicae*	Alternaria blight	Phenolics and defense related enzymes	Sood and Sohal 2012
Arabidopsis	TMV	Mosaic	Salicylic acid	Jia *et al*., 2016

Table 3: Minimum inhibitory concentration (MIC) of chitosan against several plant pathogens.

Plant pathogens	Chitosan sample	MIC(ppm)	Refrences
Bacteria			
Xanthomonas campestris	Chitosan	500	Kurita, 2006
Agrobacterium tumefaciens	Quaternary N-(benzyl) Chitosan	500	Badawy *et al*., 2014
Erwinia carotovora	Quaternary N-(benzyl) Chitosan	600	Badawy, *et al*., 2014
P.solanacearum	Chitosan	1225	Badawy, *et al*., 2016
Fungi			
Fusarium oxysporum	Chitosan	1000	Rabea *et al*., 2009
Rhizoctonia solani	Chitosan	1000	Liu *et al*., 2012
Botrytis cinerea	N-(o-methoxycinnamyl) chitosan	796	Badawyand Rabea,2013
Alternaria alternata,	N-(o-methoxycinnamyl) chitosan	672	Badawy and Rabea,2013
Phytophthora infestans	N-(o-methoxycinnamyl) chitosan	636	Badawy and Rabea,2013
Pythium debaryanum	N-(p-N-dimethylaminocinnamyl) chitosan	404	Badawy and Rabea,2013

et al., 2012). Differences in chemical structure of chitin and chitosanaccount for divergence in their antimicrobial activity that is amino group (NH_3^+) as the active functional group is essential to the antimicrobial activity of chitosan (Chung and Chen 2008). Therefore Chitin (N –acetyl glucosamine polymer) does not show any antimicrobial activity. Negatively charged compounds are present on the surface of pathogens as protein, techoic acid lipopolysaccharides and glycoproteins on the fungi, gram positive bacteria, gram negative bacteria and viral envelop respectively. Thus, the cationic chitosan molecules interact with negatively charged pathogen surfaces, disturb the cell structure, cause extensive cell surface modification and increase membrane permeability, leading to the leakage of intracellular substances and ultimately affect the pathogen vital activities (Je and Kim 2006).

ii) Membrane damage mechanism: Chitosan in activate the pathogens via a two-step mechanism, i.e., to begin with separation of the cell wall and cell membrane, followed by damage to the cell membrane(Chung and Chen 2008). The efflux of potassium ions was identified as an early response of the cell to the presence of some cationic compounds. A rapid efflux of potassium depended on the chitosan concentration. Apart from this inhibitory effect of chitosan on H^+-ATPase activity in the plasma membrane is also detected (Xing *et al*., 2015). The decrease in the H^+-ATPase's activity could incite the protons accumulation inside the cell, which would result in the inhibition of the chemiosotic driven transport that allows the H+/K+ exchange and increase the plasma membrane fluidity (García-Rincóna *et al*., 2010).

iii).Chitosan-DNA/RNA interactions: Lowmolecular weight chitosan is able topass through the cell wall (Goy *et al*., 2009), destroy intracellular components from colloidalstate to flocculation and degeneration, disrupt the normal physiological and metabolic activity, or directlyinterfere with genetic materials (Issam *et al*., 2005). Phosphate groups in the main chain of nucleic acid (DNA/ RNA). Amino groups of chitosan that possess positive charges attract the negatively charged phosphate groups of DNA/RNA. Chitosan, bind with DNA and inhibit transcription and translation through penetration toward the nuclei of the pathogens.

iv).Metal chelation capacity of chitosan: Disruption of the barrier present on outer membrane is the first step in antimicrobial activities. Metal ions such as $Ca^{++}$and Mg^{++} are present on the fungal and bacterial cell wall and cell membraneand give the stability to their cellular structures. Chitosan have chelating property and chelate the metal ions, essential nutrients and trace elements from pathogen cell surfaces led to release of metal ions and pathogen get collapsed ultimately.

v). Deposition onto the microbial surface: Chitosan can deposit onto the pathogen cell surface or between the cell

Table 4: Listing of antimicrobial action of chitosan

Chitosan sample	Microorganism	Mode of action	Refrences
Chitosan	*E. coli* and *S. aureus*	Enzyme and nucleotide leakage	Chung and Chen 2008
Chitosan	*Colletotrichum falcatum*	Mycelia growth and sporulation inhibitor	Katiyar *et al*., 2016
Chitosan Nanoparticles	*Candida albicans, Fusarium solani* and *Aspergillus niger*	Plasma membrane disruption, Inhibitor of DNA and RNA synthesis	Ing *et al*., 2012
Chitosan	*P. syringae pv. tomato*	Chelation of metal ions and cell aggregation	Mansilla *et al*., 2013
Chitosan	*E. coli* and *S. aureus*	Electrostatic interaction	Liu *et al*., 2004
N-(o-methoxy-cinnamyl) Chitosan	*Alternaria alternata,*	Inhibition mycelia growth and sporulation	Badawy and Rabea, 2013
Chitosan	*P. infestans*	Defence enzyme	Atia, *et al*., 2015
N-(p-N-dimethyl aminocinnamyl) Chitosan	*Pythium debaryanum*	Inhibition mycelia growth and sporulation	Badawy and Rabea, 2013

wall and cell membrane, form a polymer film. Surface of chitosan-treated cells, were covered by numerous vesicular structures in outer membrane and an additional layer of material, causing thickened cell envelop (Helander *et al.*, 2001). The thickened cell envelope causes blockage of nutrients flow from entering the cell, as well as the extracellular transport of metabolite excretion.

Delicate fruits like strawberry, Cherries and grapes are highly susceptible for post harvest decay caused by post harvest fungal pathogens like grey mold (*Botrytis cinerea*), Blue mold (*Penicillium expansum*), Brown mold (*Monilinia* spp) and soft rot fungi (*Rhizopus stolonifer*). Romanazz (2010) has been reported 1% Chitosan is effective for inhibition of post harvest pathogen and it enhances the self life by their dual action as it reduces the growth of decay causing fungi, and it stimulate resistance responses in host tissues. With this double effectiveness; chitosan can be considered as the first compound of a new class of plant protection products.

Apart from elicitor and antimicrobial activity chitosan act as substrate for biocontrol fungi *Trichoderma* and act synergistically (Sajeesh *et al.*, 2016). Chitosan coating on potato tuber reduces storage loss and accelerates germination due to improvement in sprout thickness (Ravichandran *et al.*, 2015).

CONCLUSION AND FUTURE PERSPECTIVE

Chitosan is a natural biodegradable compound, possessing broad-spectrum resistance elicitor activities with antimicrobial effects, has potential in agriculture sector with regard to controlling plant diseases. It is compatible with other integrated pest management technology and may offset the wide use of chemical pesticides to provide safe food in ecologically balanced environment. Antibacterial and antifungal potency of chitosan in food systems has been affirmed by several studies. Its unique preiperties, resistance inducer, antimicrobial ability and good film-forming properties, separated it from other degradable polymers.

Although much work has been done but there is many unclear points in mode of action of chitosan in relation to antimicrobial and elicitor properties. Actual target sites on cell surface for chitosan binding are still unidentified. Modification on physical property of chitosan is also demanded which improves their solubility in water and enhances immunity triggering effect. Compatibility of chitosan with other biocontrol agents and chemical pesticides needs to be work so that it will give better field results.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Council of Scientific and Industrial Research, New Delhi for their support.

REFERENCES

Arcidiacono, S. and Kaplan D.L. (1992). Molecular weight distribution of chitosan isolated from *Mucor rouxii* under different culture and processing conditions. *Biotechnology and Bioengineering*, 39: 281–286.

Atia , M.M.M., Buchenauer, H., Aly, A.Z. and Abou-Zaid, M.I. (2005). Antifungal Activity of Chitosan AgainstPhytophthorainfestans and Activation of Defence Mechanisms in Tomato to Late Blight. *Biological Agriculture and Horticulture,* **23***: 175-197.*

Awadalla O.A. and Mahmoud Y.A.G. (2005). New chitosan derivatives induced resistance to *Fusarium* wilt disease through phytoalexin (Gossypol) production. *Sains Malaysiana*, **34:** 141-146.

Badawy M.E.I. and Rabea E.I. (2013). Synthesis and structure-activity relationship of N(cinnamyl) chitosan analogs as antimicrobial agents. *International Journal of Biological Macromolecule.* **57**:185-92.

Badawy M.E.I., Rabea E.I. and Taktak N.E.M. (2014). Antimicrobial and inhibitory enzyme activity of *N*-(benzyl) and quaternary *N*-(benzyl) chitosan derivatives on plant pathogens. *Carbohydrate Polymers,* **111**: 670-682.

Badawy M.E.I., Rabea E.I., Taktak N.E.M. and Nouby M.A.M.E. (2016). The Antibacterial Activity of Chitosan Products Blended with Monoterpenes and Their Biofilms against Plant Pathogenic Bacteria. *Scientifica* 2016: 1-10.

Chatterjee, S., Adhya, M., Guha, A.K., and Chatterjee, B.(2005). Chitosan from Mucor rouxii: Production and physico-chemical characterization. *Boichemistry* **40**: 395-400

Chatterjee, S., Chatterjee, S., Chatterjee, B., Chatterjee, P. and Guha K. (2009). Influence of plant growth hormones on the growth of *Mucor rouxii* and chitosan production. *Microbiological Research,* **64:** 347-351.

Chung, Y.C. and Chen, C.Y. (2008). Antibacterial characteristics and activity of acid-soluble chitosan. *Bioresource Technology,* **3:** 2806–2814.

Eilenberg, H., Pnini-Cohen, S., Rahamim, Y., Sionov, E., Segal, E., Carmeli, S. and Zilberstein,A. (2010). Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant Nepenthes khasiana. *Journal of Experimental Botany,* **61:** 911–922.

Feng, Y. and Xia W. (2011). Preparation, characterization and antibacterial activity of water soluble O-fumaryl-chitosan. *Carbohydrate Polymers*, **83:**1169-1173.

Garcia-Rincóna, J., Vega-Pérezb, J., Guerra-Sánchezb, M.G., Hernandez Lauzardoa, A.N., Pena-DíazcA. and Velazquez-Del Vallea, M.G. (2010). Effect of chitosan on growth and plasma membrane properties of Rhizopus stolonifer (Ehrenb.:Fr.) Vuill. *Pesticide Biochemistry and Physiology* **97:** 275–278.

Goy, R.C., Britto, D. and Assis, O.B.G. (2009). A review of the antimicrobial activity of chitosan. *Polimeros,* **19:** 241–247.

Guilli, M.E., Hamza, A., Clément C., Ibriz, M., and Barka E.A. (2016). Effectiveness of Postharvest Treatment with Chitosan to Control Citrus Green Mold. *Agriculture.* **6:** 2- 12.

Hadwiger, L.A. (2013). Multiple effects of chitosan on plant systems: solid science or hype. *Plant Sciences,* **208:** 42–49.

Hafdani, F.N. and Sadeghinia, N. (2011). A review on application of chitosan as a natural antimicrobial. *International Scholarly and Scientific Research and Innovation*, **5:** 46-50.

He, G., Chen, X., Yin, Y., Zheng,, H., Xiong, X. and Du, Y. (2011). Synthesis, characterization and antibacterial activity of salicyloyl chitosan. *Carbohydrate Polymers*, **83:**1274-1278.

Helander, I.M., Nurmiaho-Lassila, E.L., Ahvenainen, R., Rhoades, J. and Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*, **71:** 235–244.

Hsu, S.H., Chang, Y.B., Tsai, C.L., Fu, K.Y., Wang, S.H. and Tseng, H.J. (2011). Characterization and biocompatibility of chitosan nanocomposites. *Colloid Surface*, **85:** 198–206.

Hu, Y., Du, Y., Yang, J., Kennedy, J. F. Wang, X. and Wang, L. (2007). Synthesis, characterization and antibacterial activity of guanidinylated chitosan. *Carbohydrate Polymers*, **67:** 66-72.

Ing, L.Y., Zin, N.M., Sarwar, A. and Katas, H. (2012). Antifungal Activity of Chitosan Nanoparticles and Correlation with Their Physical Properties. *International Journal of Biomaterials*, **2012:** 1-9.

Iriti M., Vitalini S., Di Tommaso G., D'Amico S., Borgo M and Faoro F. (2011). New chitosan formulation prevents grapevine powdery mildew infection and improves polyphenol content and free radical scavenging activity of grape and wine. *Australian Journal of Grape Wine Research*, **17:** 263–269.

Issam, S.T., Adele, M.G., Adele, C.P., Stephane, G. and Veronique, C. (2005). Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. *Journal of Food Science*, **70:** 100–104.

Je, J.Y. and Kim, S.K. (2006). Chitosan derivatives killed bacteria by disrupting the outer and inner membrane. *Journal of Agricultural and Food Chemistry*, **54:** 6629–6633.

Jia, X., Meng, Q., Zeng, H., Wang, W and Yin, H. (2016). Chitosan oligosaccharide induces resistance to *Tobacco* mosaic virus in Arabidopsis via the salicylic acid-mediated signalling pathway.*Scientific Report*, **6:** 1-12.

Kareem, A.E.F. and Haggag, W.M. (2014). Chitosan and citral alone or in combination for controlling early blight disease of potato plants under field conditions. *Research journal of Pharmaceutical, Biological, Chemical. Science*, **5:** 941-949.

Katiyar, D., Hemantaranjan, A. and Singh B. (2016). Antifungal Activity of Chitosan and Chitooligosaccharides against Red Rot of Sugarcane. *Journal of Mycology and Plant pathology*, **46:** 224 – 228.

Kurita K. (2006). Chitin and chitosan: functional biopolymers from marine crustaceans. *Marine Biotechnology*, **8:** 203-226.

Li, X., Shi, X. Wang, M. and Du, Y. (2011). Xylan chitosan conjugate –A potential foodpreservative. *Food Chemistry*, **126:** 520- 525.

Liu, H., Du, Y., Wang, X. and Sun, L. (2004). Chitosan kills bacteria through cell membrane damage. *International Journal of Food Microbiology*, **95:** 147-55.

Liu, H., Tian W., Li, B.,Wu, G., Ibrahim, M., Tao, Z., Wang, Y., Xie G., Li, H. and Sun, G. (2012). Antifungal effect and mechanism of chitosan against the rice sheath blight pathogen, *Rhizoctonia solani*. *Biotechnology Letters*, **34:** 2291–2298.

Liu, X.F., Guan, Y.L., Yang, D. Z., Li Z. and Yao, K. D. (2000). Antibacterial action of chitosanand carboxymethylated chitosan. *Journal of Applied Polymer Science*, **79:**1324-1335.

Mansilla, A.Y., Albertengo, L., Rodríguez, M.S., Debbaudt, A., Zúñiga, A., and Casalongue C.A. (2013). Evidence on antimicrobial properties and mode of action of a chitosan obtained from crustacean exoskeletons on *Pseudomonas syringae* pv. tomato DC3000. *Applied Microbiology Biotechnology*, **15:** 6957-6966.

Raafat D. and Sahl H.G. (2009). Chitosan and its antimicrobial potential – a critical literature survey. *Microbial Biotechnology*, **2:** 186-201.

Rabea, E.I., Badawy, M.E.I., Steurbaut, W. and Stevens C.V. (2009). In vitro assessment of N (benzyl) chitosan derivatives against some plant pathogenic bacteria and fungi. *European Polymer Journal*, **45:** 237–245.

Ravichandran G., Venkatasalam E.P. and Manorama K. (2015). Role of bioactive polymer coatingon potato microtuber storage and field performance. *Indian Journal of Horticulture*, **72:** 107-113.

Romanazzi, G. (2010). Chitosan treatment for the control of postharvest decay of table grapes, strawberries and sweet cherries. *Fresh Produce*, **4:** 111-115.

Runarsson, O.V., Holappa, J., Nevalainen, T., Hjalmarsdottir, M., Järvinen, T., Loftsson, T., Einarsson, J. M., Jonsdottir, S., Valdimarsdottir, M. and Masson, M. (2007). Antibacterial activity of methylated chitosan and chitooligomer derivatives: Synthesis and structure activity relationships. *European Polymer Journal*, **43:** 2660-2671.

Sajeesh P.K. (2015). Cu-Chi-Tri: A triple combination for the management of late blight disease of potato (*Solanum tuberosum* L.). PhD Thesis GBPUA&T, Pantnagar, (India).

Sajeesh P.K. Bhardwaj, N.R. Balodi, R. and Kumar J. (2016). Field evaluation of triplecombination of copper, chitosan and *Trichoderma* for management of late blight disease of potato under hill condition. *Advances in Life Sciences*, **5:** 2771-2778.

Silva, T.H., Alves, A., Ferreira, B.M., Oliveira, J.M., Reys, L.L., Ferreira, R.J.F., Sousa, RA., Silva, S. S., Mano, J.F. and Reis, R.L. (2012). Materials of marine origin: a review on polymers and ceramics of biomedical interest. *International Materials Reviews*, **57:**276-306.

Sood N. and Sohal B.S. (2012). Studies in relation to chitosan and its potential to enhance host resistance in *Brassica juncea* L. against *Alternaria* blight. *Plant Disease Research*, 144-148.

Tang, H., Zhang, P., Kieft, T. L., Ryan, S. J., Baker, S. M., Wiesmann, W. P. and Rogelj, S.(2010). Antibacterial action of a novel functionalized chitosan-arginine against Gram negative bacteria. *Acta Biomaterialia*, **6:** 2562-2571.

Vallapa, N., Wiarachai, O., Thongchul, N., Pan, J., Tangpasuthadol, V., Kiatkamjornwong, S. and Hoven, V. P. (2011). Enhancing antibacterial activity of chitosan surface by heterogeneous quaternization. *Carbohydrate Polymers*, **83:** 868-875.

Xing, K., Zhu, X., Peng, X and Qin, S. (2015). Chitosan antimicrobial and eliciting properties forpest control in agriculture: a review. *Agronomy for Sustainable Development*, **35:** 569-588.

Yang, T. C. Chou, C. C. and Li C. F. (2005). Antibacterial activity of N-alkylated disaccharide chitosan derivatives. *International Journal of Food Microbiology*, **97:** 237– 245.