Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 2281-2286
Received: 10-07-2019
Accepted: 12-08-2019

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Chitosan: A novel bioactive compound for mangement of plant diseases: A review

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Abstract
Chitosan has received much interest for potential wide application in agriculture due to its excellent biocompatibility, biodegradability and bioactivity. Chitosan is, a linear polysaccharide composed of randomly distributed b-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) is an environmental friendly product. Chitin can be easily obtained from shellfish waste. The chitosan molecule triggers defense responses within the plant, leading to the formation of physical and chemical barriers against invading pathogens. Chitosan has been used to stimulate the immunity of plants to protect plants against microorganisms. This protection leads stimulation of plant growth. Chitosan affects various physiological responses like plant immunity, defense mechanisms involving various enzymes such as, phenylalanine ammonium lyase, polyphenol oxidase, tyrosine ammonia lyase and antioxidant enzymes viz., activities superoxide dismutase, catalase and peroxide against adverse conditions. Recent studies have shown that chitosan induces mechanisms in plants against various biotic (fungi, bacteria, and insects) and abiotic (salinity, drought, heavy metal and cold) stresses and helps in formation of barriers that enhances plant's productivity. Chitosan has also used as a promising postharvest treatment for fruits due to its natural character, antimicrobial activity and elicitation of defense responses. It has also been shown that chitosan promotes vegetative growth and enhances various processes in developing flower buds. Chitosan treatment have been reported that chitosan increased shoot length, root length, shoot dry weight and relative water content under salinity stress.

Keywords: Chitosan, oligochitosan, physiological responses, defense mechanism, plant immunity, antimicrobial activity

Introduction
In recent years there has been an increasing interest in finding alternatives to chemical bactericides and fungicides considered as safe, and with negligible risk to human health and environment. Among these strategies, some satisfactory results have been reported using natural compounds such as chitosan (Muzzarelli 1983) [43]. A biopolymer of chitosan is one of the most abundant naturally occurring amino-polysaccharides of glucosamine and N-acetylglucosamine and it has attracted attention because of its unique physiochemical characteristics and biological activities (No & Meyers 1997) [46]. Chitosan is a linear polysaccharide composed of randomly distributed b-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) is an environmental friendly product. Chitosan is a polymer of high molecular weight, similar to cellulose. The only difference between chitosan and cellulose is the amine (–NH2) group in the position C-2 of chitosan instead of the hydroxyl (–OH) group found in cellulose. Chitin can be easily obtained from shellfish waste and insects, as well as certain other organisms such as fungi (mostly zygomycetes), algae, and yeast. Chitosan is one of the most common polymers found in nature. The biggest producers of chitosan are situated in nations with expansive shellfish preparing enterprises. Agricultural chitosan is generally provided by organizations in Korea, China, India and Norway. Chitosan go about as a plant safeguard sponsor got from deactylation of chitin. Chitosan has been accounted for to initiate multifaceted disease resistance. This natural biopolymer is known for its unique properties like, biodegradability, nontoxicity and antimicrobial activity, thus popularizing its use as an elicitor molecule for different host-pathogen interaction studies. Moreover, various studies have shown that chitosan has antifungal and antimicrobial effects (Kumar *et al.* 2004) [35]. Chitosan is a chemically stable, white to pale yellow powder or flake. Chitosan has a solid positive charge, which is the premise of its utilization as a "sticking" agent (i.e., a glue adjuvant). The decidedly charged atoms hold fast to contrarily charged pesticides and plant surfaces. The chitosan particle triggers resistance reactions inside the plant, prompting the development of physical and substance hindrances against attacking pathogens. Chitosan has been used to stimulate the immunity of plants to protect plants against microorganisms.

This protection leads stimulation of plant growth (Bautista *et al.* 2003) [5]. The method of activity of chitosan is to stimulate natural defence response systems frameworks in treated plants. Poly-D-glucosamine ties to contagious receptor destinations, mirroring an assault by parasitic spores. This thusly results in signs being sent to the cores of the plant and activating signs which evoke numerous hereditary and organic reactions, including the creation of phytoalexins (hostile to microbial mixes delivered in plants), went for repressing contaminations. Both chitin and chitosan have demonstrated antiviral, antibacterial, and antifungal properties, and have been explored for many agricultural uses. They have been utilized to control disease or reduce their spread, to chelate nutrient and minerals, preventing pathogens from accessing them, or to enhance plant innate defenses. When used to enhance plant defenses, chitin and chitosan induce host defense responses in both monocotyledons and dicotyledons. These responses include lignification (Barber *et al.*, 1989) [3], ion flux variations, cytoplasmic acidification, membrane depolarization and protein phosphorylation (Felix *et al.*, 1998) [12], chitinase and glucanase activation (Roby and Kaku *et al.*, 1987, 1997) [53], phytoalexin biosynthesis (Ren and Yamada *et al*., 1992, 1993) [65], generation of reactive oxygen species, biosynthesis of jasmonic acid, and the expression of unique early responsive and defense-related genes (Minami *et al.* 1996, Nishizawa *et al*. 1999, Takai *et al*, 2001) [42, 45, 56]. In addition, chitosan was reported to induce callose formation (Conrath *et al.*, 1989) [8], proteinase inhibitors (Walker *et al*., 1984) [61], and phytoalexin biosynthesis (Hadwiger *et al.*, 1980) [19] in many dicot species. The response to chitin, chitosan, and derived oligosaccharides varies with their acetylation degree.

Uses of chitosan in agriculture

Chitosan effects on plant response were first characterized as an elicitor. The unique physiological and biological properties of chitosan have led to its use in various industries, including agriculture, as a coating material for fruits, seeds and vegetables (Lee *et al*., 2005) [36]. Chitosan stimulate plant immune systems, protect plants against attack by microorganism's growth and crop productivity. chitosan coating has the potential to prolong storage life and to control decay of many fruit such as strawberries (Hernandez *et al.* 2006) [20], and papaya (Sivakumar *et al*. 2005) [55]. Chitosan treatment have been reported to prolongs storage life and controls decay of cucumber, carrot, apple, citrus, kiwifruit, peach, pear, strawberry, and sweet cherry (Ben *et al*,. 2003) [6]. One of the unique characteristics of chitosan-based coating is that it is a carrier for incorporating functional ingredients, such as antimicrobial agents and nutraceuticals (Park and Zhao 2004) [47]. Xianling *et al*., (2002) [63] 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.

Influence of chitosan on plant invulnerability

The potent effect of chitosan on plant diseases is due to its antimicrobial properties and plant innate immunity elicitation activity. Chitosan has also used as a promising postharvest treatment for fruits due to its natural character, antimicrobial activity and elicitation of defense responses (Katiyar *et al*., 2014) [28]. Chitosan has been used to control postharvest diseases of many fruits such as pear (Yu *et al.*, 2008) [68], strawberry (Ge *et al.*, 2010) [14], table grape (Meng *et al*., 2008), tomato (Badawya and Rabeab 2009) [2], citrus and longan (Jiang and Li 2001) [25]. Chitosan is one of the most important elicitors. Researchers have proved that it elicit plant defense response to a broad spectrum of phytopathogens, including plant virus (Terry and Joyce 2004) [57]. At present many experimental results have proved that chitosan can inhibit viral infection (Pospieszny 1997) [49]. However, the antiviral activity mainly depends on the molecular structure of chitosan, especially on the molecular weight. It was found that low molecular-weight chitosan (oligochitosan) is more effective in suppressing infection of the tobacco mosaic virus (TMV) in tobacco plants, and antiviral activity of chitosan increased as its molecular weight decreased (Kulikov *et al.*, 2006). It has been reported that chitosan activated systemic acquired resistance against tobacco necrosis virus (TNV) ensued from a programmed cell death, which was similar to that occurred in the hypersensitive response (Iriti *et al.*, 2006) [24]. Chitosan application can also elicit callose deposition, which has a partial effect on inhibiting virus spreading. The activation of a Ca^{+2} dependent callose synthase is one of the most rapid, effective cell responses to chitosan treatment. Calcium is one of the most important second messenger in innumerous plant signaling pathways. Chitosan, which has been shown to induce elevation of (Ca^{+2}) activates plant defense responses through the calcium signaling pathway (Klusenser *et al*., 2002) [30].

Chitosan in defense mechanisms

Nichoson *et al.,* (1992) 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. Chitosan has been demonstrated to induce defense mechanism in tomato, cucumber (Ben *et al.*, 2003) [6], and rose shrubs (Wojdyla 2004) [62]. Chitosan induces the accumulation of phytoalexins resulting in antifungal responses and enhanced protection from further infections (Vasyukova *et al.*, 2001) [60]. Spraying with chitosan has been shown to significantly reduce severity of leaf spot disease and increase the length of inflorescences in *Dendrobium missteen*. Chitosan treatment increased polyphenol oxidase (PPO) activity in disease resistant cultivars (Raj *et al*., 2006) [51]. Chitosan may be involved in the signaling pathway for the biosynthesis of phenolics. It has been shown that chitosan can induce chitinase and chitosanase, which are members of a group of plant pathogenesis related (PR) proteins. These PR proteins can degrade the cell walls of some phyto pathogens and consequently may play a role in host plant defense systems (Dixon *et al*., 1994) [9]. Further studies have shown that chitosan induces the expression of various genes involved in plant defence responses such as genes encoding PAL and protease inhibitors (Vander *et al.*, 1998) [58]. Genetic studies have shown that chitosan may involve jasmonic acid (JA) pathways, since transcription activation of genes encoding PAL and protease inhibitors are induced by both JA and chitosan (Farmer and Ryan 1992) [10]. Therefore, the antifungal action of chitosan seems to comprise more than one mode of action by which chitosan affects fungal cell wall biosynthesis and/or alteration of the ability of pathogens to infect and/or its ability to increase plant resistance.

Yue *et al*., (2001) [69] 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.

Chitosan as plant guard activator

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*., (1983) [37] 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.

The term ‘plant defense booster’ applies to a group of compounds, which act by triggering various physiological and morphological responses within the plant that help to stimulate natural defense mechanisms. Quang *et al.* (2006) [50] reported that positive effects of chitosan on barely plants depend on the molecular weight of the applied chitosan. Chitosan biosynthesis in fungi (Hadwiger 2009) [17] starts with the sugar nucleotide, uridine diphosphate N-acetyl-Dglucosamine (UDP-GlcNAc) that is incorporated into chitin. Two enzymes, chitin synthetase and chitin deacetylase produce chitosan (Kafetzopoulos *et al.* 1993) [26] The commercial production of chitosan originates as crustacean chitin. Chitosan as a polycationic polymer elicits cellular changes (Yin *et al*., 2010) [67], viz, membrane depolarization oxidative burst, influx and exit of ions such as Ca2 kinases, DNA alteration, mRNA, PR proteins, phytoalexins; lignifications and callose deposition.

Roles of chitosan in plant defense

Chitosan may be involved in the signaling pathway for the biosynthesis of phenolics. It has been shown that chitosan can induce chitinase and chitosanase, which are members of a group of plant pathogenesis related (PR) proteins.

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Chitosan enhance the germination

Ma *et al.* (2014) [39] reported that oligochitosan promoted wheat growth in terms of germination capacity, root length seedling height and increase in root activity. Shao *et al.* (2005) [54] reported that seed soaked with chitosan increased the germination percentage of maize seed. Manjunatha *et al.* (2008) [40] reported that seed priming with chitosan enhanced seed germination and seedling vigour in pearl millet. Seed soaked with chitosan increased germination rate, length and weight of hypocotyls and radical in rapeseed. Zeng and Luo (2012) [70] reported that chitosan has excellent film forming property, making it easy to form a semipermeable film on the seed surface, which can maintain the seed moisture and absorb the soil moisture, and thus it can promote seed germination priming with 0.2 % chitosan was found to be superior to all other concentrations. Researcher have reported that chitosan increased shoot length, root length, shoot dry weight and relative water content under salinity stress. Similar results were reported by Ma *et al*. (2012) [38], who reported that wheat seeds treated with chitosan showed higher growth than control under salinity stress. It is suggested that chitosan triggers the defensive mechanisms in plants, stimulates root growth and induces certain enzymes such as chitinases, pectinases and glucanases (Hien 2004) [21]. Chitosan promotes plant growth through increasing the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure (Guan *et al.,* 2009) [15].

Antimicrobial activity

Plant pathogenic bacteria and fungi are considered economically important around the world. They induce decay on a large number of economically important agricultural crops during the growing season and during postharvest storage. Control of this disease is especially important and can be achieved by synthetic pesticides. However, there are growing environmental problems caused by bactericides and fungicides, especially by synthetic products (Houeto *et al.* 1995) [23]. The antimicrobial activity of chitosan and its oligosaccharide derivative has been recognized and is considered to be one of the most important properties. Allan *et al.,* (1979) [1] 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., (*2002)

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. However, Zheng *et al.,* (2003) [72] 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*., (2005) [66] Hirano *et al*., (1989) [22] 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 alternate* than high–molecular– weight chitosan.

References

1. Allan CR, Hadwiger LA. The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp. Mycol. 1979; 3(3):285-287.
2. Badawya MEI, Rabeab EI. Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. Post-harvest Biology Technology. 2009; 51:110-117.
3. Barber MS, Bertram RE, Ride JP. Chitin oligosaccharides elicit lignification in wounded wheat leaves. Physiol. Mol. Plant Pathol. 1989; 34:3-12.
4. Barka AE, Eullaffroy P, Cle´ment C, Vernet G. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Reports. 2004; 22:608-614.
5. Bautista BS, Hernandez LM, Bosquez ME, Wilson CL. Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. Crop Protection. 2003; 22:1087-1092.
6. Ben BN, Ardi R, Pinto R, Aki C, Fallik E. Controlling gray mould caused by Botrytis cinerea in cucumber plants by means of chitosan. Crop Protection. 2003; 22:285-290.
7. Carson R. Silent Spring. Houghton Miffin, Boston, 1995, 368.
8. Conrath U, Domard A, Kauss H. Chitosan-elicited synthesis of callose and of coumarin derivatives in parsley cell suspension cultures. Plant Cell Rep. 1989; 8:152-155.
9. Dixon RA, Harrison MJ, Lamb CJ. Early events in the activation of plant defenses. Annual Review of Phyto-patholgy. 1994; 32:479-510.
10. Farmer EE, Ryan CA. Octadecanoid precursors of jasmonic acid activate the synthesis of wound inducible proteinase inhibitors. Plant cell. 1992; 4:129-134.
11. Farmer EE, Ryan CA. Octadecanoid precursors of jasmonic acid activate the synthesis of wound inducible proteinase inhibitors. Plant cell. 1992; 4:129-134.
12. Felix G, Baureithel K, Boller T. Desensitization of the perception system for chitin fragments in tomato cells. Plant Physiol. 1998; 117:643-650.
13. Felix G, Regenass M, Boller T. Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalinization, changes in protein phosphorylation, and establishment of a refractory state. Plant J. 1993; 4:307-316.
14. Ge LL, Zhang HY, Chen KP, Ma LC, Xu ZL. Effect of chitin on the antagonistic activity of Rhodotorula glutinis against Botrytis cinerea in strawberries and the possible mechanisms involved. Food Chemistry. 2010; 120:490-495.
15. Guan YJ, Hu J, Wang XJ, Shao CX. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. Journal of Zhejiang University Science. 2009; 10:427-433.
16. Hadwiger LA. Pea–*Fusarium solani* interactions contributions of a system toward understanding disease resistance. Phytopathology. 2008; 98:372-379.
17. Hadwiger LA. Localization predictions for gene products involved in non-host resistance responses in a model plant/fungal pathogen interaction. Plant Science. 2009; 177:257-265.
18. Hadwiger LA. Multiple effects of chitosan on plant systems: Solid science or hype. Plant Science. 2013; 208:42-49.
19. Hadwiger LA, Beckman J. Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiol. 1980; 66:205-211.
20. Hernandez MP, Almenar E, Ocio MJ, Gavara R. Effect of calcium dipsand chitosan coatings on postharvest life of strawberries (*Fragaria* x *ananass*). Postharvest Biology Technology. 2006; 39:247-253.
21. Hien QN. Radiation processing of chitosan and some biological effects. Radiation Processing of Polysaccharides. 2004; 1:67-73.
22. Hirano S, Nagao N. Effects of chitosan, pectic acid, lysozyme, and chitinase on the growth of several phytopathogens. Agri and Biol Chem. 1989; 53(11):3065-3066.
23. Houeto P, Bindoula G, Hoffman JR. Ethylene bisdithicarbamates and ethylene thiourea: possible human health hazards. Environmental Health Perspective. 1995; 103:568-573.
24. Iriti M, Sironi M, Gomarasca S, Casazza AP, Soave C, Faoro F. Cell death- edited antiviral effect of chitosan in tobacco. Plant Physiology and Biochemistry. 2006; 44:893-900.
25. Jiang Y, Li Y. Effect of chitosan coating on postharvest life and quality of longan fruit. Food Chemistry. 2001; 73:139-143.
26. Kafetzopoulos D, Martinou A, Bouriotis V. Bioconversion of chitin to chitosan: Purification and characterization of chitin deacetylase from *Mucor rouxii*. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90:2564-2568.
27. Kaku H, Shibuya N, Xu P, Aryan AP, Fincher GB. *N*-acetylchitooligosaccharide elicitor expression of a single 1,3-*β*-glucanase gene in suspension-cultured cells from barley (*Hordeum vulgare*). Physiol. Plant. 1997; 100:111-118.
28. Katiyar D, Hemantaranjan A, Singh B, Bhanu NA. A future perspective in crop protection: Chitosan and its oligosaccharides. Advances in Plants Agricultural Research. 2014; 1:(06).
29. Kikuyama M, Kuchitsu K, Shibuya N. Membrane depolarization induced by *N*-acetylchitooligosaccharide elicitor in suspension-cultured rice cells. Plant Cell Physiol. 1997; 38:902-909.
30. Klusenser B, Young JJ, Murata Y, Allen GJ, Mori IC, Hugouvieux V. Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in Arabidopsis guard cells. Plant Physiology. 2002; 130:2152-2163.
31. Kohle H, Jeblick W, Poten F, Blaschek W, Kauss H. Chitosan-elicited callose synthesis in soybean cells as a Ca^{+2} dependent process. Plant Physiol. 1985; 77:544-551.
32. Kuchitsu K, Kosaka H, Shiga T, Shibuya N. EPR evidence for generation of hydroxyl radical triggered by *N*-acetylchitooligosaccharide elicitor and a protein

phosphatase inhibitor in suspension-cultured rice cells. Protoplasma. 1995; 188:138-142.
33. Kuchitsu K, Yazaki Y, Sakano K, Shibuya N. Transient cytoplasmic pH changes and ion fluxes through the plasma membrane in suspension cultured rice cells triggered by *N*-acetylchitooligosaccharide elicitor. Plant Cell Physiol. 1997; 38:1012-1018.
34. Kulikov SN, Chirkov SN, Il'ina AV, Lopatin SA, Varlamov VP. Effect of the molecular weight of chitosan on its antiviral activity in plants. Applied Biochemistry and Microbiology. 2006; 42:200-203.
35. Kumar MNVR, Muzzarelli RAA, Muzzarelli C, Sashiwa H, Domb AJ. Chitosan chemistry and pharmaceutical perspectives. Chemical Reviews. 2004; 104:6017-6084.
36. Lee YS, Kim YH, Kim SB. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. Horticulture Science. 2005; 40:1333-1335.
37. Loschke DC, Hadwiger LA, Wagoner W. Comparison of mRNA populations coding for phenylalanine ammonia lyase and other peptides from pea tissue treated with biotic and abiotic phytoalexin inducers. Physiol Plant Pathol. 1983; 23(1):163-173.
38. Ma LJ, Li YY, Yu CM, Wang Y, Li XM, Li N. Alleviation of exogenous oligo-chitosan on wheat seedlings growth under salt stress. Protoplasma. 2012; 249:393-399.
39. Ma LJ, Li YY, Yu CM, Wang Y, Li XM, Li N. Germination and physiological response of wheat (*Triticum aestivum*) to pre-soaking with oligochitosan. International Journal of Agricultural Biology. 2014; 16:766-770.
40. Manjunatha G, Roopa KS, Prashanth GN, Shekar SH. Chitosan enhances disease resistance in pearl millet against downy mildew caused by *Sclerospora graminicola* and defence-related enzyme activation. Pest Management Sciences. 2008; 64:1250-1257.
41. Meng XH, Li BQ, Liu J, Tian SP. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. Food Chemistry. 2008; 106:501-508.
42. Minami E, Kuchitsu K, He DY, Kouchi H, Midoh N, Ohtsuki Y, Shibuya N. Two novel genes rapidly and transiently activated in suspension-cultured rice cells by treatment with N-acetylchitoheptaose, a biotic elicitor for phytoalexin production. Plant Cell Physiol. 1996; 37:563-567.
43. Muzzarelli RAA. Chitin and its derivatives: New trend of applied research. Carbohydrate Polymer. 1983; 3:53-75.
44. Nicholson RL, Hamerschmidt R. Phenolic compounds and their role in disease resistance. Annu Rev. Phytopathol. 1992; 30:369-389.
45. Nishizawa Y, Kawakami A, Hibi T, He DY, Shibuya N, Minami E. Regulation of the chitinase gene expression in suspension-cultured rice cells by *N*-acetylchitooligosaccharides: differences in the signal transduction pathways leading to the activation of elicitor-responsive genes. Plant Mol. Biol. 1999; 39:907-914.
46. No HK, Meyers SP. Preparation and production of chitin and chitosan. In: Muzzarelli R.A.A., Peter M.G. (eds): Chitin Handbook. Grottammare, European Chitin Society, 1997, 475-489.
47. No HK, Park NY, Lee SH. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. International Journal of Food Microbiology. 1992; 74(1-2):65-72.
48. Nojiri H, Sugimori M, Yamane H, Nishimura Y, Yamada A, Shibuya N, Kodama O, Murofushi N, Ohmori T. Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. Plant Physiol. 1996; 110:387-392.
49. Pospieszny H. Antiviroid activity of chitosan. Crop Protection. 1997; 16:105-106.
50. Quang LL, Naotsugu N, Masao T, Tomoko N. Enhancement of plant growth activity of irradiated chitosan by molecular weight fractionation. Radioisotopes. 2006; 55:23-27.
51. Raj SN, Sarosh BR, Shetty HS. Induction and accumulation of polyphenol oxidase activities as implicated in development of resistance against pearl millet downy mildew disease. Functional Plant Biology. 2006; 33:563-571.
52. Ren YY, West CA. Elicitation of diterpene biosynthesis in rice (*Oyza sativa* L.) by chitin. Plant Physiol. 1992; 99:1169-1178.
53. Roby D, Gadelle A, Toppan A. Chitin oligosaccharides as elicitors of chitinase activity in melon plants. Biochem. Biophys. Res. Comm. 1987; 143:885-892.
54. Shao CX, Hu J, Song WJ, Hu WM. Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. Agriculture and Life Science. 2005; 31:705-708.
55. Sivakumar D, Sultanbawa Y, Ranasingh N, Kumara P, Wijesundera RLC. Effect of combined application of chitosan and carbonate salts on the incidence of anthracnose and on the quality of papaya during storage. Journal of Horticulture Sciences Biotechnology. 2005; 80:447-452.
56. Takai R, Hasegawa K, Kaku K, Shibuya N, Minami E. Isolation and analysis of expression mechanisms of a rice gene, EL5, which shows structural similarity to ATL family from *Arabidopsis*, in response to N-acetylchitooligosaccharide elicitor. Plant Sci. 2001; 160:577-583.
57. Terry LA, Joyce DC. Elicitors of induced disease resistance in postharvest horticultural crops: A brief review. Postharvest Biology and Technology. 2004; 32:1-13.
58. Vander P, Kjell MV, Domard A, El-Gueddari NE, Moerschbacher BM. Comparison of the ability of partially N-acetylated chitosans and oligosaccharides to elicit resistance in wheat leaves. Plant Physiology. 1998; 118:1353-1359.
59. Vander P, Kjell MV, Domard A, El-Gueddari NE, Moerschbacher BM. Comparison of the ability of partially N-acetylated chitosans and oligosaccharides to elicit resistance in wheat leaves. Plant Physiology. 1998; 118:1353-1359.
60. Vasyukova NI, Zinoveva LI, Il´inskaya EA, Perekhod GI, Chalenko NG, Il´ina AV. Modulation of plant resistance to diseases by water-soluble chitosan. Applied Biochemistry and Microbiology. 2001; 37:103-109.
61. Walker-Simmons M, Ryan CA. Proteinase inhibitor synthesis in tomato leaves. Plant Physiol. 1984; 76:787-790.
62. Wojdyla AT. Chitosan (biochikol 020 PC) in the control of some ornamental foliage diseases. Commun. in Agril. and Appl. Biol. Sci. 2004; 69:705-715.

63. XianLing JG, YingPing M, ZhiMei L. Effect of chitosan on physiological and biochemical characteristic of seed germination and seedling of mulberry (*Morus alba*). Acta Sericologica Sinica. 2002; 28(3):253-255.
64. Xu QJ, Nian YG, Jin XC, Yan CZ, Liu J, Jiang GM. Effects of chitosan on growth of an aquatic plant (*Hydrilla verticillata*) in polluted waters with different chemical oxygen demands. Jn. of Environ. Sci. 2007; 19:211-221.
65. Yamada A, Shibuya N, Kodama O. Akatsuka, T. Induction of phytoalexin formation in suspension cultured rice cells by *N*-acetylchitooligosaccharides. Biosci. Biotechnol. Biochem. 1993; 57:405-409.
66. Yang TC, Chou CC, Li CF. Antibacterial activity of N–alkylated disaccharide chitosan derivatives. Int. J Food Microbiol. 2005; 97(3):237-245.
67. Yin H, Zhao X, Du Y. Oligo-chitosan a plant disease vaccine—A review. Carbohydrate Polymer. 2010; 82:1-8.
68. Yu T, Wang LP, Yin Y, Wang YX, Zheng XD. Effect of chitin on the antagonistic activity of *Cryptococcus laurentii* against *Penicillium expansum* in pear fruit. International Journal of Food Microbiology. 2008; 122:44-48.
69. Yue DYZ, Zhi MZ, Yong GQ. Effect of chitosan on physiological activities in germinating seed and seedling leaves of maize. Jn. of Habei. 2001; 15(4):9-12.
70. Zeng D, Luo X. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. Journal of Soil Science. 2012; 2:282-288.
71. Zhao Y, Tu K, Su J, Tu S, Hou Y. Heat treatment in combination with antagonistic yeast reduces diseases and elicits the active defense responses in harvested cherry tomato fruit. Journal Agriculture Food Chemistry. 2009; 57:7565-7570.
72. Zheng LY, Zhu JF. Study on antimicrobial activity of chitosan with different molecular weights. Carbohydrate Polymers. 2003; 54(4):527-530.