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Review Article
Chitosan for Eco-friendly Control of Plant Disease

Oliul Hassan and Taehyun Chang

Division of Ecology and Environmental System, College of Ecology and Environmental Sciences, Kyungpook National University, 37224 Sangju-si, Gyeongsangbuk-do, Republic of Korea

Abstract
Due to the high environmental risk of chemical pesticides, biological control of plant diseases with bio-pesticides are highly encouraged and recommend. Chitosan and oligochitosan are the well-known biological control agent for its nontoxic, biodegradable and biocompatible properties. Chitosan is considered as the most abundant natural polymer with a dual effect: It controls pathogenic microorganisms by preventing growth, sporulation, spore viability, germination and disrupting cell and inducement of different defense responses in host plant inducing and/or inhibiting different biochemical activities during the plant-pathogen interaction. Chitosan has been assayed for control numerous pre and post-harvest diseases of many crops. Chitosan also has the positive effect of enriching biodiversity in the rhizosphere. Meteorological effect on chitosan is little evaluated. For achieving the goal of sustainable agriculture, chitosan will become a popular plant protectant.

Key words: Chitosan, ecofriendly, biopesticides, resistance, biodiversity

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Corresponding Author: Taehyun Chang, Division of Ecology and Environmental System, Kyungpook National University, 37224 Sangju-si, Gyeongsangbuk-do, Republic of Korea Tel: +82-54-530-1204 Fax: +82-54-530-1209

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INTRODUCTION

Chemical pesticide is of increasing concern as their negative impact on human health and environment[1,2]. The broad spectrum pesticides may cause problems by targeting beneficial organisms and repeated use of these synthetic pesticides leads to loss of biodiversity[3,4]. Some pesticides are not biodegradable and persistence in environment from few to many years for these reasons, they are responsible for soil, water and a wide range of environmental pollution[5-7]. Some pesticides bio-accumulated in the food chains which is in consequence affecting the human health[8,9]. There is lots of evidence of the emerging resistance to fungicides due to the indiscriminate use of synthetic fungicides. In these circumstances control of pest with the bio-pesticides is the better alternative to the synthetic ones as the bio-pesticides have good efficacy, low residues and little or no toxicity to non-target organisms and human, causes low environmental pollution and several other advantages[10].

Chitin and its derivative, chitosan have been known worldwide for its potential as a biocontrol agent. After cellulose, the second most abundant and important natural biopolymer is chitin in the world[11]. Chitin has a variety of applications such as in medical science, chemical science and agricultural industry. In medical science, chitin has been used as a wound dressing material, drug carrier and anti-cancer drug etc., while in chemical science it has been used to prepare an affinity chromatography column and to immobilized enzyme etc.[11]. It has been used as a food additive and bio-pesticides in the agricultural industries[12]. The most important properties of chitosan such as biodegradability, nontoxicity, biocompatibility and fungicidal effect[11,13].

A lot of biological control agents, including natural product (derived from microorganism, higher plant and animal) and microorganisms (fungi, bacteria, virus nematodes and protozoa)[10,14] have been used to control the plant diseases and to inhibit the growth of plant pathogens. Chitosan has some advantages over other biocontrol agent not only its, potential to control plant diseases but also its ability to induce resistance in the host plants[15] and enhance biodiversity in the rhizosphere[16].

The objectives this study were to summarized the efficacy of chitosan and oligo-chitosan on controlling plant diseases, mechanism of induction resistance in plant and influence on microbial community in rhizosphere.

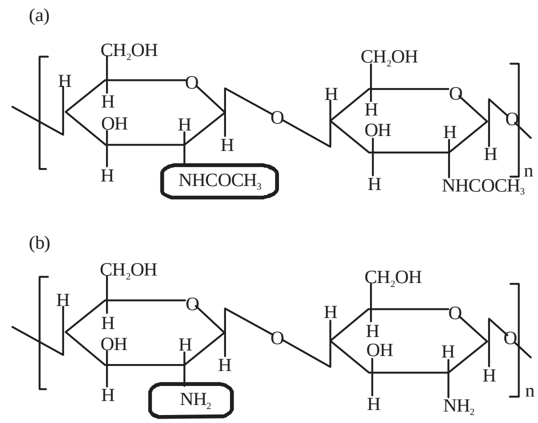

Fig. 1(a-b): Structures of (a) Chitin and (b) Chitosan[12]

CHEMICAL STRUCTURE AND FUNCTION OF CHITOSAN

Chitosan is the derivatives of chitin. It is prepared by deacetylation of chitin to varying degree, although the deacetylation is almost never complete. When the deacetylation of chitin is reaching below 50% (depending on the origin of chitin), the product is called chitosan, it became soluble in acidic aqueous media[11,17]. To convert chitin to chitosan, chitin is N-deacetylated by enzymatic preparation or chemical process. In the chemical process, acids or alkalis (NaOH) are used to deacetylate chitin[17,18]. In case of enzymatic preparation, chitin deacetylases (EC 3.5.1.41) is used to catalyze the hydrolysis of N-acetamido bonds in chitin to convert it to chitosan[17]. The structures of chitin and chitosan[12] are shown in Fig. 1.

Due to biological (biodegradable, nontoxic and biocompatibility) and chemical (reactive amino group, hydroxyl groups, positive charge, etc.)[19] properties, the chitosan has a broad range of industrial applications.

Table 1 summarizes the main application of chitosan in different industries. In this text, we will give emphasis on the use of chitosan in agriculture as elicitors, bio fungicides and modifier of plant-microbial interaction etc.

In vitro ANTIMICROBIAL ACTIVITY

***In vitro* antifungal activity:** There are several techniques that have been used to understand the antimicrobial activities of chitosan. *In vitro* antifungal activities of chitosan against several genera of fungi have been investigated and documented by several studies. Most of the study revealed that the chitosan affects mycelium growth, sporulation, morphology and molecular organization of fungus.

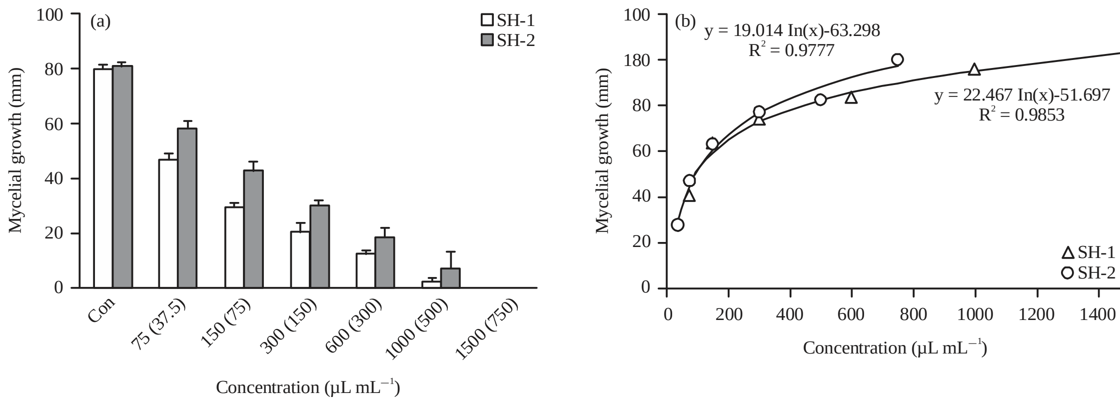

Fig. 2(a-b): Effect chitosan preparation concentrations on the rate of mycelial growth and inhibition in V-8 juice agar medium amended with SH-1 (75 μg a.i. mL^{-1} total glucosamine to 1,500 μg a.i. mL^{-1} in V-8 juice agar) and SH-2 (37.5 μg a.i. mL^{-1} total glucosamine to 750 μg a.i. mL^{-1} in V-8 juice agar) after 10 days of inoculation, (a) Effect of mycelial radial growth and (b) Control value of mycelial radical growth[43]

Table 1: Pottential application of chitosan in different field

Major field	Potential applications
Medical and pharmaceutical	Hydrophobic drug carrier, encapsulating nutrients, fibers, fabrics , artificial organ, membrane, burn treatment, wound dressing, corneal contact lens, etc.[20-22]
Agriculture	Laminated film, seed coating, potting mixture, fungicides, elicitors, etc.[23,24]
Water and waste treatment	Heavy metal trapper, odor reducer, flocculant, etc.[25,26]
Biotechnology	Emulsifier, chelator, immobilize and pattern biomolecules, graphitic carbon nanocapsules, tungsten carbide, etc.[27]
Food and beverages	Food additive, preservative, protective coating for fruits, food dyes and nutrients, etc.[28-30]
Cosmetics and toiletries	Lotions, cream, nail lacquers, hair additives soap, cleansing milk, face peel, lipstick, foot powder, tooth pest and mouth wash[31]

Chitosan effectively inhibits radial mycelium growth, sporulation, spore germination and elongation of *Fusarium* spp.,[32-34], *Rhizopus* spp.,[35,36], *Phytophthora* spp.,[37,38], *Botrytis* spp.,[39,40] and *Alternaria* spp.,[41] etc. Meng *et al.*[42] demonstrated the inhibition rate of mycelium growth of fungi depend on the concentration of chitosan and its derivatives, oligochitosan. Growth inhibition of mycelium increases with the increasing concentration of chitosan preparation SH-1 (75 μg a.i. mL^{-1} total glucosamine to 1,500 μg a.i. mL^{-1} in V-8 juice agar) and SH-2 (37.5 μg a.i. mL^{-1} total glucosamine to 750 μg a.i. mL^{-1} in V-8 juice agar) after 10 days of inoculation (Fig. 2)[43].

In almost all cases, spore-producing fungi produce lower spore when treated with chitosan than untreated fungi. Moreover, in some reports complete sporulation inhibition was observed after treating with chitosan. In contrary to sporulation inhibition, chitosan stimulates sporulation of *Penicillium digitatum* and *Alternaria alternate*[44,45]. The researchers explained this phenomenon by the responses of these fungi against the chitosan induced stress.

The inhibitory effect of chitosan depends on the type of solvent. Chitosan dissolved in lactic acid show the best inhibitory effect as compared to dissolved in formic acid and acetic acid[33]. Some studies have reported the antifungal activities of chitosan depend on the incubation period. The growth inhibition of *F. oxysporum* f. sp. *radicis-lycopersici* decreases with the increased incubation period when treated with chitosan at low concentration (1.0 and 2.0 mg mL^{-1})[46] while for *Aspergillus niger*, incubation time is very important for chitosan efficacy[47]. Efficacy of chitosan also depended on the type of chitosan. Low Molecular Weight (LMW) chitosan has the highest inhibitory effect on *Rhizopus stolonifera*, *Botrytis cinerea* and *Penicillium expansum*[39,48] while High Molecular Weight (HMW) chitosan show better efficacy on *F. oxysporum* f. sp. *vasinfectum*, *Alternaria solani* and *Valsa mali*[49]. All fungus species are not similarly sensitive to chitosan[35]. Chitosan (0.5 and 1.5%) appears to be more effective in inhibiting mycelium growth of *F. oxysporum* than *P. digitatum* and *R. stolonifera*[44]. The chitosan could be formulated and applied as a natural antifungal agent into nanoparticles form, to enhance its antifungal activity. Ing *et al.*[50] reported that chitosan nanoparticles prepared from low molecular weight and high molecular weight chitosan have better inhibitory activity against *Candida albicans*, *F. solani*. Chitosan silver nanoparticles inhibit conidia germination of *Colletotrichum gloeosporioides* more effectively than chitosan alone[51]. Special characters of the chitosan nanoparticles such as small and compact particle

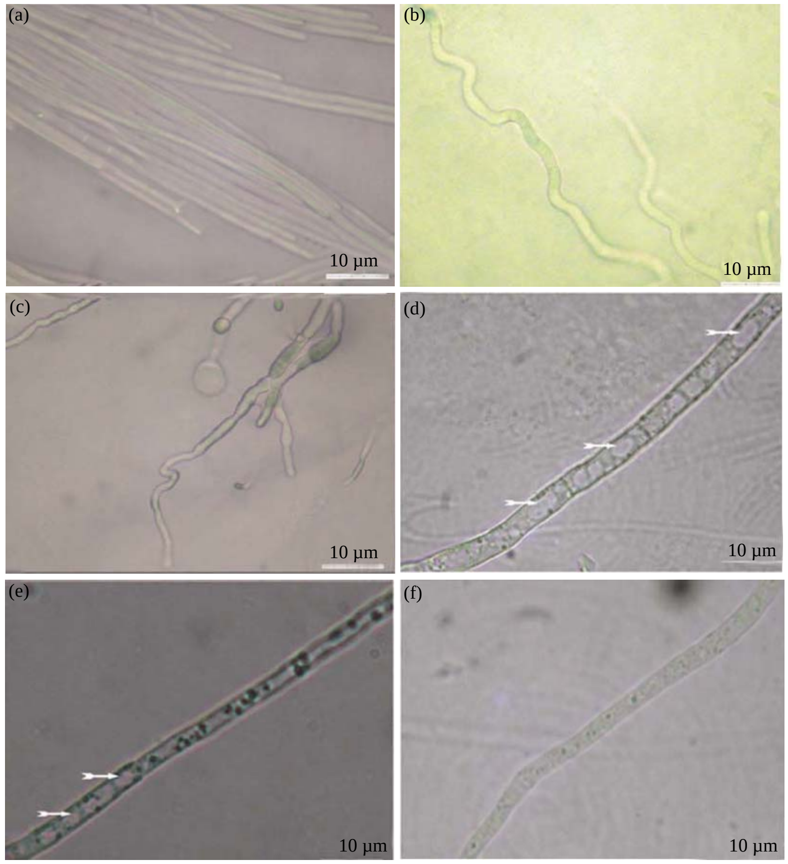

Fig. 3(a-f): Effect of chitosan on fungal morphology (a) Hyphal agglomeration, (b-c) Abnormal shapes, (d-e) Large vesicles in the mycelia (arrows) and (f) Normal hyphal growth in control[34]

as well as high surface charge are responsible for higher antifungal activities than sole chitosan polymer[50].

Effect of chitin, chitosan and its oligomers on fungal morphology is well documented. Scanning electron microscope analysis reveal that inhibition of fungal growth in response to chitosan and its oligomers was accompanied with excessive mycelial branching, abnormal shapes, swelling and hyphae size reduction in *Fusarium* spp.[32,34,46,52], *B. cinerea*, *R. stolonifera*, *A. alternate* and *Penicillium expansum*[35,53,54]. Chitosan also responsible for cytological alteration, protoplasm dissolution and large vesicle of fungus[34] shown in Fig. 3.

***In vitro* antibacterial activity:** As like as fungi, bacteria also highly sensitive to the chitosan and its derivatives. Most of the chitosan antibacterial reports are on human disease causing bacteria such as *Escherichia coli*, *Staphylococcus aureus* and certain *Bacillus* species. Since chitosan shows the bactericidal activity against a variety of human disease-causing bacteria, it could be expected that chitosan may protect the plant from bacterial diseases. Recently, some researchers revealed that chitosan has strong *in vitro* antibacterial activities against plant pathogenic bacteria such as *Streptomyces scabies*[55], *Xanthomonas*[56], *Pseudomonas syringae*[57], *Pseudomonas fluorescens*[58], *Agrobacterium tumefaciens*[41], *Erwinia*[41], *Burkholderia seminalis*[59], *Acidovorax citrulli*[60] and *Acidovorax avenae*[61]. Inhibitory activity of chitosan against bacteria depends on, concentration[41,61] molecular weight of chitosan[62] type of bacteria (Gram-positive and Gram-negative)[63], type of solvent[41], incubation period and abiotic factors[64]. From these studies, it is clear that chitosan could be used as potential bacteria controlling agent.

***In vitro* antiviral activity:** Despite chitosan is not being a component of the virus, chitosan has been shown to control plant viral diseases. Suppression of viral infection was reported with the tobacco mosaic and necrosis viruses, potato virus X, peanut stunt virus, cucumber mosaic virus and alfalfa mosaic virus[65-67]. It is hypothesized that chitosan can inhibit viral infection by inducing hypersensitive response, decreasing viability of phage bacterial cells, neutralizing the infectivity of phage particle and blocking the virulent phage replication[68].

***In vitro* nematicidal and anti-protozoal activity:** Nematicidal as well as the anti-protozoal activity of chitosan and its derivatives has not been sufficiently studied. From the 1980s onward, a number of studies suggested that chitosan is effective to control plant pathogenic nematode population[69-71]. Chitinous amendments result in significant reduction in egg hatching, the viability of larvae and adult of the plant pathogenic nematode species *Meloidogyne arenaria*[68], *Meloidogyne incognita*[72], *Meloidogyne javanica*[73] and *Heterodera glycines*[70]. The nematicidal activity of chitosan depends on the concentration, molecular weight of chitosan. Khalil and Badawy[72] suggested that the chitosan at low molecular weight may serve as a natural nematicide. A few studies have demonstrated that application of chitosan biomolecule is promising option for treatment trichomoniasis[74,75]. Deacetylated chitosan oligosaccharide significantly decreases the viability of *Trichomonas vaginalis*[74] and *Trichomonas gallinae*[75]. Still, a long way to go to make conclusive statements on chitosan as the antiprotozoal agent.

USE OF CHITOSAN FOR CONTROLLING PLANT DISEASE (*In vivo* STUDY)

Control of *Fusarium* spp.: *Fusarium* spp., is a very important plant pathogen causes disease in a wide range of plant, leading significant economic loss and mycotoxin in food and animal feed. At least 81 of 101 economically important crops are affected by *Fusarium* spp., infection. Chitosan provides a satisfactory level of pant-protection against *Fusarium* spp., as compared to chemical pesticide[33,76,77]. To control pathogens, chitosan has been utilized in seed, soil amendment and as foliar treatment[78]. *Fusarium oxysporum* the causal agent of crown and root rot disease of tomato. Foliar spraying of chitosan at concentrations ranging from 0.5-2 mg mL^{-1} markedly reduces the number of root lesions caused by the *F. oxysporum*[79]. Bhaskara Reddy *et al.*[78] reported that chitosan treatment (2-8 mg mL^{-1}) of wheat seeds reduces seed born *F. graminearum* >50%. They also suggested that chitosan treatment increase resistance in seedling and grain yield. Soil amendment with optimal concentrations of chitosan able reduced plant wilting caused by *Fusarium* spp., especially *F. acuminatum*[80]. Hadwiger and Beckman[81] found that the tissue is protected from *F. solani* f. sp., *pisi*, infection when chitosan applied to pea pod tissue. *Fusarium moniliforme* is an opportunistic microorganism associated with maize, which is able to colonize in the roots and uptake nutrient. Use of chitosan as maize seed coating agent inhibit the *F. moniliforme* growth[82]. Dry rot is an important disease of stored potato caused by *F. sulphureum*. Li *et al.*[33] reported that chitosan application at the rate of 0.5 or 1% effectively controlled the dry rot of potato tuber.

Control of *Phytopthera* spp.: According to the results of several studies, *Phytopthera* spp., is effectively controlled by chitosan. Spraying and soil drench with chitosan reduced *Phytopthera infestans*[43,83,84], *Phytophthora cactorum*[85] and *Phytophthora capsici*[85] diseases. Plant sprayed with N, O-carboxymethyl chitosan was shown to delay late blight development (*P. infestans*)[83]. Cho *et al.*[84] reported that oligochitosan suppressed the development of late blight on potato with the control value of 72%. Chang and Kim[43] after evaluating chitosan preparations (SH-1 and SH-2) on late blight suggested that SH-1 and SH-2 can be used as the natural fungicide in organic farming to increases tuber yield and to control the late blight disease. *Phytophthora cactorum* is the causal organism for crown rot in strawberry. Effect of chitosan on crown rot disease depends on the concentration of chitosan and time between application and inoculation. Disease severity decrease as the time between treatment and inoculation increased. It may take certain time interval for the plant to install its defenses. Low concentrations of chitosan were more effective against crown rot than high concentrations. *Phytophthora* root and crown rot of bell pepper caused by *Phytophthora capsicum* were better controlled when the soil of nursery bed is amended with the chitosan (0.2% w/v)[86]. *Phytophthora parasitica* is a root pathogen with a wide range of host. For example, black shank (*P. parasitica*) a devastating disease of all types of cultivated tobacco. The result of Falcon *et al.*[87] showed that partially acetylated chitosan was very effective in protecting tobacco against *P. parasitica*.

Control of *Rhizoctonia* spp. and *Sclerotinia* spp.: The potential of chitosan to suppress the diseases caused by *Rhizoctonia* spp. and *Sclerotina* spp., has been confirmed various investigations. *Rhizoctonia solani* is a soil born plant pathogen which is responsible for damping-off, sheath rot and root rot of many plant species. Acid soluble chitosan protects rice seedling from sheath blight (*R. solani*) and causes a 66-91% inhibition lesion length[88]. In other studies carried out with lettuce treated with biochikol 020 PC, (2% of chitosan) showing symptoms of damping-off (*R. solani*) was significantly lower[89].

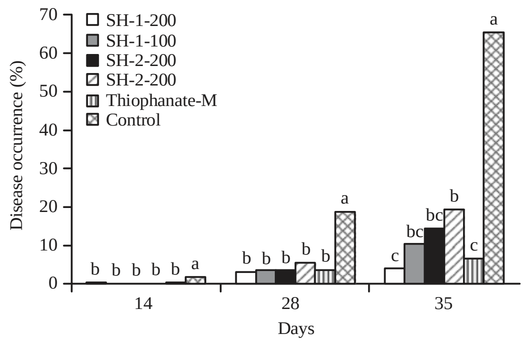

Fig. 4: Protective activity of chitosan preparations on tomato leaf mold[103]

Sclerotinia sclerotiorum is another soil born plant pathogen causing root crown and stem rot in various plant species. Application of chitosan hydrolysate at the rate of 0.2% (w/v) to carrot 3 days before inoculation reduced the size and frequency of *Sclerotinia* rot as well as induced resistance[90]. Yin *et al.*[91] found the similar result when oligochitosan when applied to oilseed rape 3 days before inoculation.

Control of *Pythium* spp., *Penicillium* spp. and *Puccinia* spp.: *Pythium* species cause diseases in a wide range of crops. As for the example root rot of cucumber and rhizome rot disease of turmeric are the very destructive disease caused by *Pythium aphanidermatum*. El Ghaouth *et al.*[92] investigated the biological activity of chitosan on *Pythium* rot of cucumber. It is found that chitosan (100-400 mg mL^{-1}) not only control the root rot by adversely affecting the growth of *P. aphanidermatum* but also stimulated physical barrier and enzymatic defense mechanism without affecting normal growth of cucumber (Fig. 4). Symptom of phytotoxicity also was not found even in higher concentration of chitosan. Anusuya and Sathiyabama[93] reported that turmeric plant treated with chitosan (0.1% w/v spraying on leaves) showed increased resistance towards rhizome rot disease caused by *P. aphanidermatum*.

Blue mould and green mould diseases (caused by several species of *Penicillium*) cause considerable economic losses on fruit and vegetable during the storing period. Coating fruits and vegetable with chitosan protect them from pathogen and increase their self-life[39,94,95]. Tomato fruits treated with 0.5 and 1% chitosan could significantly decrease blue mould caused by *P. expansum* when stored at 2°C and the effects of chitosan on this disease were increase with the increasing concentration of chitosan[39]. Wang *et al.*[95] found that chitosan significantly inhibits blue mold caused by *P. expansum* and natural decay on jujube fruit in concentration dependent manner. Coating citrus (*Murcott tangor*) fruit with chitosan significantly reduced postharvest decay caused by *Penicillium. digitatum* and *Penicillium italicum* in respect of molecular weight (high or low)[95].

Peanut (*Arachis hypogaea*) is one of the popular and high demanding crops all over the world for it nutritional value. The study conducted on peanut infection with *Puccinia arachidis* indicated that chitosan (1000 ppm) has a good controlling effect on leaf rust disease. Chitosan reduced the number of lesions and lesion diameter when applied before inoculation[96].

Control of *Botrytis* spp., *Colletotrichum* spp. and other fungal species: *Botrytis cinerea* Pers., a necrotroph plant pathogen, causes gray mould disease in over 200 plant species. Many studies showed that chitosan and its derivatives have the ability to inhibit the development of gray mould in different plant species such as cucumber[97], grapes[98] and tomato[99] etc. The chitosan has the ability decrease in the gray mould disease index from 3.5 in the control plants to 0.45 when spraying on cucumber plants before 24 h of inoculation[97]. Xu *et al.*[98] evaluated the antifungal activity of grapes seed extract and chitosan, alone or combined, against fungal rot (*B. cinerea*) of the grapefruits. It is found that GSE and chitosan have synergistic effect and treatment with 1% chitosan plus 0.1% GSE also significantly reduced gray mold incidence flowed by treatment with 1% chitosan coating. As a biocontrol agent chitosan also found effective against tomato gray mold. About 0.2% of chitosan solution significantly inhibited tomato gray mold[99].

Colletotrichum anthracnose (*Colletotrichum* spp.) is one of the most economically important plant disease causing serious economic loss. A wide range of crops is affected by this diseases. The studies that have been conducted to determine the effects of chitosan on *Colletotrichum* anthracnose revealed that it (chitosan) has satisfiable controlling effect. More than 60% control of anthracnose on papaya fruit is achievable when applied before *C. gloeosporioides* inoculation[100]. Significant reduction of anthracnose disease after coating tomato fruits and grape berry with 2.5% chitosan reported by Munoz *et al.*[101]. Chitosan also induces resistance in common bean plant hence effectively protected it (common bean) from anthracnose caused by *Colletotrichum lindemuthianum*[102].

We evaluated the disease control efficacy of two chitosan preparations (SH-1 and SH-2) against tomato leaf mold caused by *Fulvia fulva* was under plastic greenhouse conditions[103]. We made SH-1 preparation by mixing chitosan 2 g, chitooligosaccharide 20 g, wood vinegar 20 mL, rice vinegar 40 g and SH-2 by mixing chitosan 2 g, chitooligosaccharide 20 g, wood vinegar 40 mL and rice vinegar 20 g. In this study,

Table 2: Effect of chitosan on viral infection in different 'Plant-virus' systems[114]

Plant species	Virus	Effect of chitosan	Rate of inhibition[a]
Phuseolus vulgaris	ALMV-L	Reduction in the number of local lesion	++++
Phaseolus vulgaris	TNV		++++
Chenopodium quinoa	TNV		++
Chenopodium quinoa	CMV		+++
Nicotiana tabacum var. Samsun NN	TMV		+++
Nicotiana tabacum var. Xanthi nc	TMV		++
Nicotiana glutinosa	TMV		++
Nicotiana paniculata	PSV		++
Phaseolus vulgaris	ALMV-S	Reduction in the number of systemically infected	++++
Phaseolus vulgaris	PSV	plants	++++
Pisum sativum	ALMV		+++
Pisum sativum	PSV		+++
Lycopersicum esculentum	PVX		+++

[a]Rate of inhibition: ++: 20-50%, +++: 50-75%, ++++: 75-100%

we applied chitosan preparation prior to the occurrence of leaf mold on tomato plants and disease severity was rated at different days after three applications at 7 days interval. It is found that both SH-1 and SH-2 formulations displayed potential disease control activity in every case and which was comparable to synthetic thiophanate-M (Fig. 4).

Similarly chitosan potential of controlling fungal diseases such as kernel rot (*Aspergillus flavus*) in preharvest maize[104], root rot (*Bipolaris sorokiniana*) of wheat[65], disease of pears cause by *A. kikuchiana* and *Physalospora piricola*[42], post-harvest pathogenic fungi (*Rhizopus stolonife*, *Aspergillus niger*) in grapes[105], brown rot (*Monilinia fructicola*) in peach fruit[106], downy mildew (*Sclerospora graminicola*) of pearl millet[107] and downy mildew (*Elsinoe ampelina*) and anthracnose (*Plasmopara viticola*) in grapevines[108] was also reported.

Control of Bacterial, viral and nematode disease: Experimental (*in vivo*) evidence has shown the bactericidal (kill bacteria directly) and bacteriostatic (stop bacteria from growing) ability of chitosan. Soil drench with chitosan significantly reduced *Ralstonia* wilt (*Ralstonia solanacearum*) incidence by 72% when the tomato is grown in the greenhouse. Regardless of application of chitosan, it improves the normal growth of tomato[109].

The different species of *Xanthomonas* are responsible for the many destructive diseases in different plant species such as leaf spot of tomato, leaf blight and leaf sreak in rice, bacterial blight of cotton and citrus canker. For evaluating the antibacterial effect of chitosans with different molecular weights on *X. gardneri* (leaf spot) development and tomato plants protection. Coqueiro and Di Piero[110] sprayed chitosan (1 mg mL^{-1}) onto plants 72, 48 or 24 h before inoculation with *X. gardneri*. It is found that chitosans (low molecular weight, medium molecular weight and commercial) controlled tomato bacterial spot irrespective of molecular weight and reduced disease severity by 70% when applied 72 h before inoculation. Foliar spray of a commercial formulation of chitosan (Armour-Zen®) has the ability to reduce the incidences of *Xanthomonas vesicatoria* in greenhouse and field grown tomato plants[111]. The strong antibacterial activity against leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) of rice also reported by Li *et al*.[112].

Broccoli (*Brassica oleracea* L. var. *italica*) is one of the popular vegetables in the world. Growing of broccoli has been seriously hampered by the bacterial head rot (*Pseudomonas fluorescens*) disease. Regardless of application time (pre or post), six different combinations of chitosan solutions significantly reduced the disease incidence and the lesion diameter of broccoli inoculated with *P. fluorescens*[56]. Elexa, the commercial formulation of chitosan effectively protect cucumber from the damage caused by bacterial angular leaf spot (*P. lachrymans*)[113].

To date, a few studies have reported *in vivo* viral disease control effect of chitosan. The host-virus combination, chitosan concentration and mode of its application are the chitosan efficacy determining factors. Pospieszny *et al*.[114] demonstrated that maximum 99.5-100% alfalfa mosaic virus (ALMV) inhibition is possible when 0.01-0.1% chitosan sprayed into the surface of the leaves before (1 day) inoculation of plants. It is also demonstrated that the varying effect of chitosan on viral infection in different 'Plant-virus' systems. The highest level of inhibition of viral infection was observed in bean leaves while TMV, TNV and CMV's local infection inhibition in tobacco and *C. quinoa* leaves was not so pronounced in these host (Table 2). Induction of antiviral defensive reactions may varies with the host plant species.

Tobacco Necrosis Virus (TNV) is one of the greatest threats for the cultivation of the tobacco. Chitosan reduces the virus-induced necrotic lesions by 32- 83% when applied on tobacco at the rate of 0.1%[115]. Chitosan has the ability to delay the viral diseases. In comparison to control plant, chitosan retarded tomato yellow leaf curl disease symptoms about 7-14 days.

A soil amendment with chitosan was shown to reduce nematode parameters, i.e., number of galls, egg masses, females/root system and number of juveniles/250 g soil. El-Sayed *et al.*[73] reported that the standard concentration (1:1 dilution) of LMW and HMW chitosan reduces the number of gall by 90 and 93% respectively. Nunes da Silva *et al.*[116] also reported that application of all molecular weight chitosan decreases the severity of the Pine Wilt Disease (PWD) caused by *Bursaphelenchus xylophilus* (pine wood nematode). They also reported that HMW chitosan is more effective than LMW chitosan.

MECHANISMS OF CHITOSAN AND ITS DERIVATIVES IN REDUCING PLANT DISEASES

There are a few concrete proofs regarding the anti-microbial mechanism of chitosan and its ability to elicit plant defense responses. The growing evidence is showing that the chitosan and its derivatives have a double mechanism of action: They inhibits the growth of the pathogen and they modify the plant defense responses[99,117-119]. The possible mechanisms of chitosan as a bio-pesticides is summarized in Fig. 5.

It is hypothesized that the presence of positive charge and reactive amino groups (protonated amino group on the structure of chitosan) are the base of the direct antimicrobial activities of chitosan. The electrostatic interaction between positively charged chitosan and its derivatives with negatively charged microbial cell membranes resulting in agglutination, disruption and alteration of the intracellular ultrastructure of the cell membrane and thereby causing the death organism (Fig. 6)[33,120-122].

Chung and Chen[123] reported that chitosan react on both the cell membrane and the cell wall and inactivated the pathogen via a two-step sequential mechanism: An initial separation of the cell wall from its cell membrane, followed by destruction of the cell membrane. Chitosan may interfere the function of the genetic material of the pathogen. The nucleic acid (DNA/RNA) possesses the phosphate groups in the main

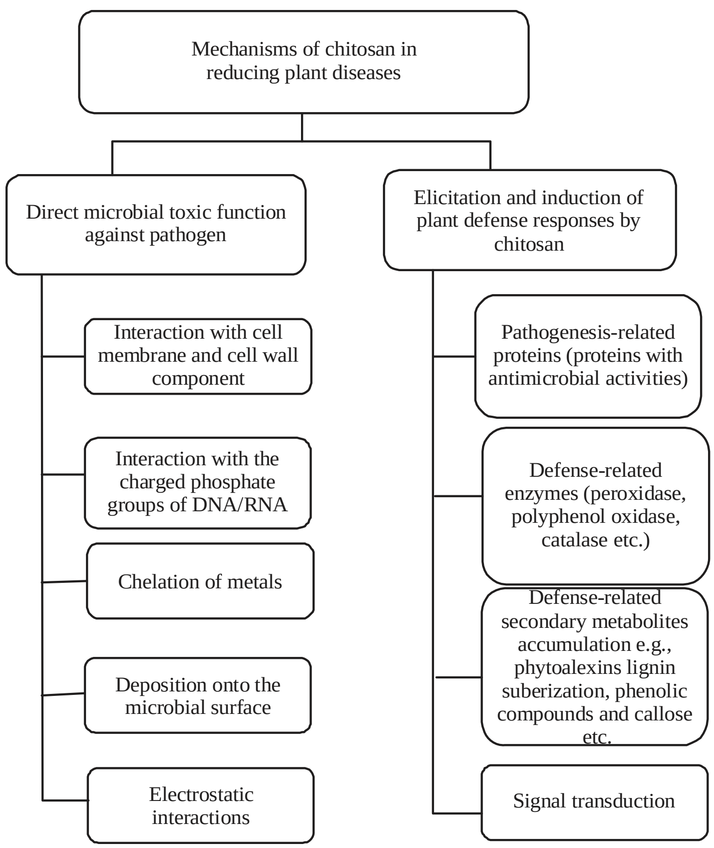

Fig. 5: Modes of action of chitosan and its derivatives as antimicrobial compounds and the ability to elicit natural plant defense responses[117]

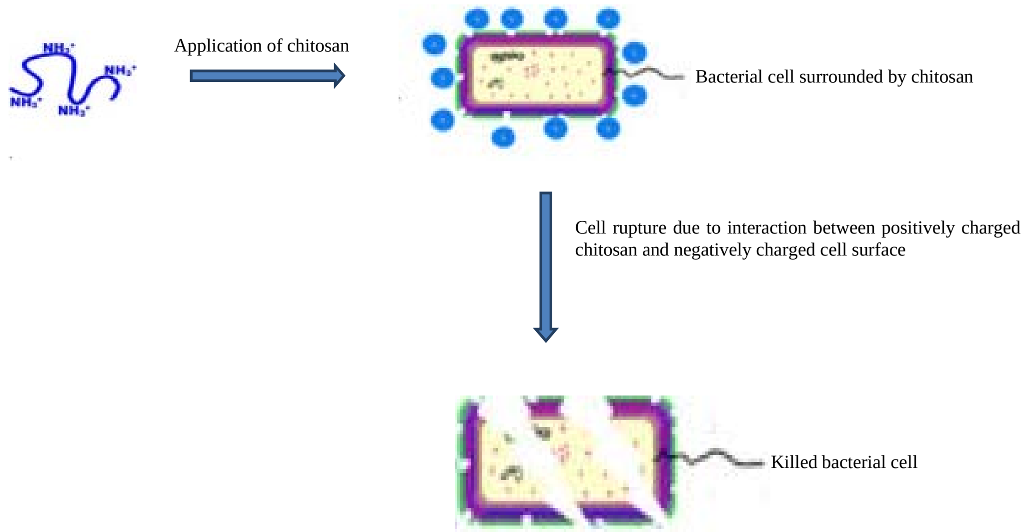

Fig. 6: Mechanism of killing of bacterial cell by chitosan[122]

chain which are negatively charged. The chitosan could penetrate cell wall[17] and bind to the negatively charged DNA and alter its confirmation in consequence block the synthesis of mRNA and essential protein of pathogen[89,121,124]. The mode of action of chitosan may also be related to its ability to chelate some essential nutrients, metal ions and trace elements necessary for the growth of bacteria and fungi[125-127].

Another possible mean of antimicrobial potential of chitosan is to deposit on the pathogen surface and the formation of a dense polymer film. This dense polymer film blocks the nutrient flow and metabolism process of microbes which are essential for their survival in nature[117].

Plant protect themselves from pathogens by developing a stunning array of structural, chemical and protein-based defenses designed to recognize invading pathogens and arrest them before they are able to cause extensive damages[128].

Some reports indicate that chitosan and its derivatives are effective elicitor and inducer of plant systemic acquired resistance to pathogens. Chitosan and oligochitosan induce hosts to produce defense-related protein[117,124], enzymes[117,129] and secondary metabolites[117]. Chitosan and its derivatives increase level and activity of glucanase in rice, tobacco, wheat, etc.[15,130,131]. It is also reported that chitosan and its derivatives increase, level and activity of chitinase, phenylalanine ammonia-lyase, peroxidase, polyphenol oxidase, catalase and superoxide dismutase in orange, tomato, cucumber, table grapes, strawberries, sweet cherries, pears and *Zanthoxylum bungeanum* (Huajiao in Chinese)[43,117-119]. All the mentioned protein and enzymes directly or indirectly degrade the pathogen.

The genes that functionally develop disease resistance are generally called pathogenesis-related genes. Some reports showed that chitosan employs multiple modes to increase pathogenesis-related gene function. As, for example, Hoat *et al.*[132] reported that chitosan and chitin strongly activated the expression of the pathogenesis-related gene (PR-10) in oat leave.

It is well accepted that plant secondary metabolites such as phytoalexins, lignin, suberization and phenolic compounds play a predominant role in plant defense system. The effect of chitosan on the accumulation of defense-related secondary metabolites in the plant tissue was studied from the 1980s when Hadwiger and Beckman[81] found chitosan at a concentration as low as 0.9 μg mL^{-1} can induce a phytoalexin accumulation in soybean pod in 24 h. These biopolymers (chitosan and its derivatives) also have the ability to enhance production and accumulation of phenolic compound (polyphenol), lignin, suberin and callose in different host plant[83,78,131,133,134]. Accumulation of the polyphenol is the plant part dependent phenomenon. As for example it is found that the content of total polyphenol in the stem was significantly increased by chitosan preparation application in the field (Fig. 7)[43].

The resistance plants have the ability of timely recognition of the plant pathogen and the rapid and fruitful activation of the defense system. The activation of defense system manifested by the Hypersensitive Response (HR) at the site of infection and activating defense in uninfected plant part[135]. The development of HR and the Systematic Acquired Resistance (SAR) govern by the combined genotypes of host and pathogen (gene for gen theory) and depend on a

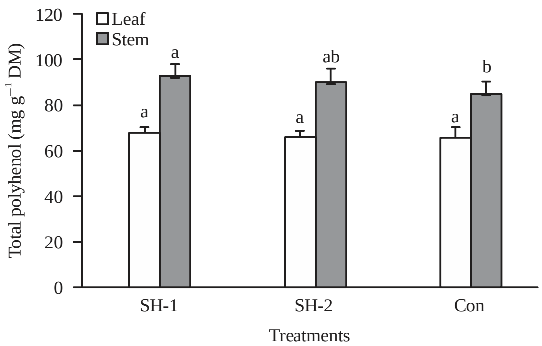

Fig. 7: Accumulation of the polyphenol is the plant part after application of chitosan preparation. SH-1 and SH-2 are the chitosan preparation[43]

complex exchange of signal occurring under given environmental condition. The signal transduction pathways composed of activation of the specific receptor on the cell membrane or located intracellular followed by transmission of the signal into the target cell mediated by one or more second messengers and create a series of physiological responses. To our knowledge, only the kinase 1 (CERK1) and the lectin are the two cell surface or membrane receptors that are likely to bind chitosan and its oligomers[136,137]. Chitosan also influences the plant's DNA conformation. Due to the high affinity for negatively charged phosphates of the DNA backbone, chitosan may alter chromatin via competition with basic nuclear proteins for DNA attachment sites that block gene transcription in turn[124,132].

On the basis of the result of several studies, Reactive Oxygen Species (ROS), Ca^{2+}, Nitric Oxide (NO), ethylene (ET), Jasmonic Acid (JA), Salicylic Acid (SA) and abscisic acid (ABA) all involved in chitosan-mediated signal pathway[117,138].

It may conclude that chitosan and its derivatives can directly destroy the pathogen and activated the plant immune system through different signaling pathways or involved in signal transduction as a regulatory molecules. Despite extensive research, the mechanism and mode of action of chitosan in reducing plant diseases have not been explicated clearly. However, the mechanisms governing the mode of action of these biopolymers yet to be studied.

ECOLOGICAL CONSIDERATION

Effect of chitosan on environment: Chitosan and its derivatives have emerged as the best ecofriendly bio-pesticides for their unique plant pathogen controlling properties, safety to human and mammals and non-toxicity to other beneficial microbes. The US Environmental Protection Agency (EPA) concluded in 2015[139] that there are no risk to the environment because chitosan has not shown toxicity in mammals and it is natural biopolymers. The National Pesticides Information Center (NPIC) database also indicates that there has been no report on environmental degradation due to the use of chitosan.

Effect of chitosan on microbial community in soils: Soil harbours most of the microorganism. Plant interact with soil microorganism in a different way that directly or indirectly influence the growth and development of the plant. Symbiotic microorganism such as mycorrhiza and rhizobia (N-fixing bacteria) promote the growth of the plant by enhancing the plant nutritional status while soil born pathogen limit the plant growth. There are other groups of microorganism which are antagonistic to soil born pathogen hence indirectly influence the plant growth. It is believed that addition of chitosan and its oligomers improves the soil structure and alter the rhizospheric environment to shift the microbial balance in favour of beneficial organisms and to the detriment of plant pathogens[24,140].

There is no substantial body of evidence that chitosan supplementation increases both population and efficacy of antagonists in the rhizosphere. Mulawarman *et al.*[141] reported that bacterial population densities of bacterial genera: *Acinetobacter* and *Pseuodomonas* increased by 8.9 and 81%, respectively when soil amended with chitosan.

Pastucha[142] isolated the greatest number of bacteria and fungi with antagonistic effect towards plant pathogens from rhizosphere soil of soybean after using chitosan three times. *Lysobacter enzymogenes* is an antagonist biocontrol agent for managing root and crown rot in cucumber caused by *Pythium aphanidermatum* (Edson) Fitzp. The addition of chitosan (depending on concentration) in soil increases cell number (cells per gram root) on the root as well as the efficacy of *L. enzymogenes*[143]. *Bacillus subtilis* is another, the most widely used biopesticide in agriculture which action against powdery mildew and number of population increases with the addition of chitosan in strawberry[144,145]. Mishra *et al.*[146] observed the substantially higher number of bacterial cells (*Pseudomonas* sp.) in tomato roots by scanning electron microscopy after the application of *Pseudomonas* sp., in combination with chitosan. It is also observed that chitosan enhance the efficacy of *Pseudomonas* sp., against tomato leaf curl virus (ToLCV) in tomato.

Mycorrhizal fungi, the most ubiquitous of root-associated plant symbioses, enhance the nutrient and water uptake efficiency of the plant. Our previous study[16] showed that

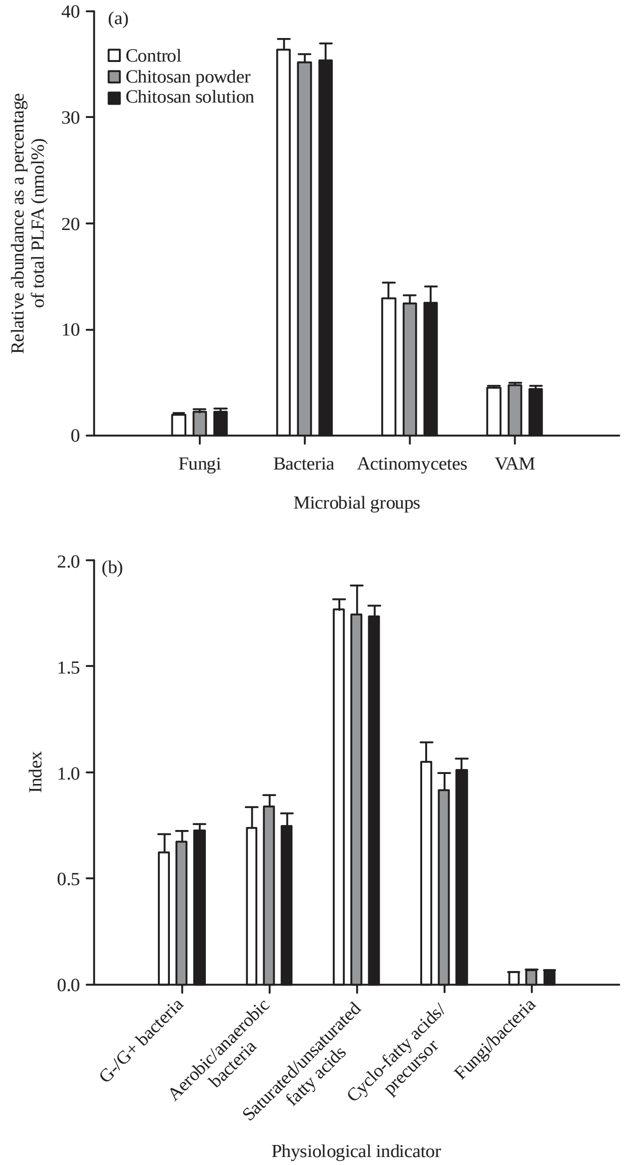

Fig. 8(a-b): Effect of chitosan on soil microbial community. Relative abundance of soil microbial groups and physiological parameter were determined by analyzing phospholipid fatty acids (PLFA) in the soils amended with chitosan powder and solution in a cucumber greenhouse at 160 days after soil amendment, (a) Mean microbial group and (b) Physiological indicator of microbes[16]

vesicular-arbuscular mycorrhizal fungi (VAM) significantly increased in soils amended with chitosan powder. It is also subsisted that chitosan powder changed the soil microbial community and the effects maintained up to 160 days after soil application (Fig. 8).

In legume nitrogen fixation process, a group of free living bacteria (rhizobia) colonized in the specialized root nodules. The addition of short-chain acetylated chitin derivatives can induce nodulation in *Medicago sativa* hence, influence the N-fixing bacterial population size and activity[147].

Effects of chitosan in stimulating beneficial soil microbial activity can be explained by following major points: (1) Chitosan may serve as nutrient (C and N) source for the microbes[142,145], (2) Chitosan may induce the chitinases producing and antagonistic gene expression in biological control agents[24,143], (3) Chitosan may induce horizontal gene transfer (natural competence) where DNA was absorbed and recombined into the chitinolytic bacterium and able to being adapted quickly to changing conditions[24], (4) Chitosan may help the antagonistic biological control agents to attack plant pathogen by disrupting the formation of biofilms produced by pathogenic microbes[24], (5) Chitinous nod (lipochito-oligosaccharides) and Myc (signal molecules) factors in growth-promoting microbes (mycorrhiza and *Rhizobium*) sensitizes plant roots to nodule formation and to interact with mycorrhizal fungi. Chitin-derivatives which are biochemically similar to nod and myc factors may induce nodulation and the formation of root connections with nitrogen-fixing bacteria and mycorrhizal fungi[24,148].

The soil microbial activities are influenced not only by chitosan but also root exudates, organic matter content soil and applied fertilizer[149,150]. Abundant occurrence of microorganisms in the soil might have been caused by the synergistic effect of chitosan and root exudates, organic matter and fertilizer. So actual effect of chitosan and its derivatives on beneficial soil microbial activities need to be discovered.

Regulation of tolerance to abiotic stresses: Abiotic stresses such as drought, soil pollution with heavy metal, heat and high and low temperature etc., adversely affect the potential production of crops. Chitosan and its derivatives based treatments can improve plant survival and performance in stressful growing environment. Chitosan increases the chlorophyll concentration and performance of photosynthesis under drought condition[151,134]. Plant need well developed roots system to survive under scare water condition. Zeng and Luo[151] reported that chitosan stimulates the root system development of wheat seedling and in consequence strengthen the capability of water absorption. Excessive transpiration is not desirable in dry condition. The concentration of ABA and JA have been found to increase in response to chitosan treatment and these hormones are well known for their ability to control stomatal aperture[23,152]. Herde *et al.*[152] observed that both ABA and JA reduce the transcription rate in the tomato plant. It is also reported that chitosan induces drought resistance in rice, drought tolerance apple, coffee and paper[23,153,154]. Bittelli *et al.*[153] found that foliar application of chitosan on pepper leaves induced partial or full closure of plant stomata, resulting in reduced water use by 26-43%.

Under the adverse environmental condition, Reactive Oxygen Species (ROS) such as peroxides, superoxide, hydroxyl radical and singlet oxygen level can increase dramatically. This may cause significant damage to the lipid cell membrane, protein and nucleic acid instead of acting as a messenger in normal cell signal transduction, cell cycling and the plant immune system. The chitosan Reactive Oxygen Species (ROS) scavenging activities were investigated by Je and Kim[155]. It is found that chitosan showed scavenging effects against hydroxyl and superoxide anion radical, the effects were 91.67 and 65.34%, respectively. These results suggest that the scavenging effect of chitosan depends on their degree of deacetylation and substituted group. Rajalakshmi *et al.*[156] also reported that chitosan and oligo chitosan possess potent antioxidant activity and hydrogen peroxide and superoxide scavenging activity.

The chelating ability of chitosan and oligo-chitosan led to the interest in their potential application to the remediation of contaminated soil and water with heavy metal, dye and accidently application of excess fertilizer and pesticides[25,26,157-159]. The presence of amino groups (-NH) in chitosan give the vital binding site for complexing with non-nutrient elemental ions, including a number of heavy metal cations[157,159]. In this way, chitosan protects the plant from the effects of heavy metals, excessive fertilizers and pesticides.

CONCLUSION

In the past few decades, chitosan and its derivatives based pesticides are gaining more attention because of their non-toxic, biodegradable, biocompatible and eco-friendly nature. Chitosan and its derivatives have been repeatedly shown very impressive results as bio-pesticide against many pests of agricultural importance which is comparable to those achieved with current synthetic pesticides and fertilizers. Chitosan is considered as the most abundant natural polymer with a dual effect: It controls pathogenic microorganisms by preventing growth, sporulation, spore viability, germination and disrupting cell and inducement of different defense responses in host plant inducing and/or inhibiting different biochemical activities during the plant-pathogen interaction. The plant can also acquire enhanced tolerance to the stressful growing environment after application of chitosan and oligochitosan. Along with these, the stimulating effect of chitosan on beneficial microorganism lead this natural product to incorporate in to the Integrated Pest Management (IPM) strategies.

There are several issues associated with the commercial use of chitosan based pesticides that are still to be investigated. Like photosensitivity of chitosan derivatives (amorphous chitosan derivatives may absorb UV spectrum) has to be managed for its long lasting effectiveness. The effective concentration and application method of chitosan products is not defined yet. The biochemical mode of action of chitosan and oligochitosan for controlling plant diseases are still limited and unclear. So, much of study is still to be done in this field.

SIGNIFICANCE STATEMENTS

The second most abundant and important natural biopolymer is chitin in the world. Chitin has been used different industries such as in medicine, chemical, cosmetics and agriculture. In the agricultural industry, these biopolymers have been using different ways including biopesticides for controlling plant diseases, bio-transformant for the production of quality food products preservative to protect foods from microbial deterioration, the chelator of waste material from food processing discards, the purifier of water and fruit juices etc. Use of chitosan as biopesticides is very effective and eco-friendly because, it is biodegradable, non-toxic and biocompatible. This study presents a comprehensive review of the antifungal action of and plant protection by chitosan.

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