



## Chitosan a New Perspective towards Biotic and Abiotic Stress Management in Agriculture: A Review

Divya Vani Sirigireddy<sup>1</sup>, Sudarshna Kumari<sup>1\*</sup>, Mantramurthy Sri Datha<sup>1</sup>, Jincy M.<sup>1</sup>, Hanuwant Singh<sup>1</sup>, Gurdeep Bains<sup>2</sup> and K. P. Singh<sup>2</sup>

<sup>1</sup>Dept. of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, Punjab (144 411), India

<sup>2</sup>Dept. of Plant Physiology, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uddam Singh Nagar, Uttarakhand (263 145), India

### Corresponding Author

Sudarshna Kumari

e-mail: sudarshnakumari89@gmail.com

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### Abstract

Plant development and crop productivity are drastically reduced worldwide due to biotic and abiotic stresses and their unexpected combinations. The various chemicals (pesticides, fertilizers, and phyto-regulators) and genetic engineering techniques employed to date to improve crop tolerance to multiple stresses have a negative influence on the environment and are time-consuming. This has accelerated efforts to find more eco-friendly ways to control plant stress. Chitosan is a biopolymer which is largely extracted from the deacetylation of chitin and appears as a viable tool to overcome these problems in search of a more environmentally acceptable solution. Due to its biocompatibility, eco-friendly and economic nature, become one of the most popular biopolymers used in agriculture. Chitosan also activates a defence mechanism by signal transduction pathway and transduces secondary molecules of hydrogen peroxide and nitric oxide to scavenge reactive oxygen species. Application of chitosan before subjecting to abiotic stresses such as drought, salt, and heat has been shown to stimulate plant growth and enhance the production of antioxidant enzymes, secondary metabolites, and abscisic acid. In drought, it helps to accumulate osmo-protectants to maintain the water potential of plant cells. On the other hand, plant responses towards chitosan are varying based on its structures, doses, developmental stages and crop type. Keeping these facts in mind this review has written with the objective to update the recent studies on chitosan, its various sources and its effective concentrations in different crops, mechanism of action against biotic and abiotic stress management to improve crop production in agriculture.

**Keywords:** Agriculture, abiotic stress, biotic stress, chitosan, problems, prospects

### 1. Introduction

Plant growth and development are severely disrupted by biotic stresses such as diseases, insects, wounding and abiotic stresses including water logging, drought, heat and heavy metal toxicity, and their erratic combination causes major yield loss globally. (He et al., 2018, Kumari et al., 2021). As the global population continues to grow and food demands increase, it is increasingly crucial to enhance the resistance of crops to various stresses (He et al., 2018). To boost a plant's tolerance to stresses, various methods have so far been tested. Particularly, the application of various insecticides, herbicides, fertilisers, and plant hormones, has led to an increase in resistance. However, the widespread application of these synthetic chemicals in cultivation has a negative effect on our planet because they accumulate in the soil, water, and aquatic

life (Malerba et al., 2016). This has prompted researchers to look for more environmentally acceptable ways to alleviate plant stresses. A major component of the cell wall of fungi and the exoskeleton of arthropods, and their deacetylated derivative chitosan appear to be possible solutions to these challenges. Chitosan, a naturally existing linear polysaccharide comprised of D-Glucosamine and N-Acetyl-D-Glucosamine (Malerba and Cerana., 2020). Chitosan also activates defence mechanism by signal transduction & introduces signalling molecules (NO) nitric oxide and (H<sub>2</sub>O<sub>2</sub>) hydrogen peroxide to scavenge reactive oxygen species. Chitosan enhances plant defence mechanisms by promoting photochemical processes and stimulating enzymes associated with photosynthesis. When applied to plant foliage, chitosan triggers the hydrolysis of peptidoglycan in microbes, resulting in microbial death.



Additionally, chitosan pretreatment promotes faster plant growth when applied prior to abiotic stressors. This pretreatment also promotes the synthesis of advantageous secondary metabolites, enzymatic antioxidants, and ABA (abscisic acid) as demonstrated in the study by (Pongprayoon et al., 2022). During drought conditions, chitosan aids in the accumulation of osmo-protectants, thereby preserving the water potential of plant cells. Additionally, it induces alterations in the cellular molecular biology, physiological process and biochemistry in plants. However, the specific responses of plants to CT vary depend on factors such as the structure of chitosan, its concentration, plant species, and developmental stages (Kumari et al., 2021). Chitosan also induced plant defence by invigorating photochemistry and photosynthetic enzymes. Under biotic stress foliar application of chitosan leads to hydrolysis of peptidoglycan in microbes and leads to death. Scientists have proposed a variety of applications for chitosan in the field of agriculture due to its antimicrobial (anti-fungicidal, anti- bacteriocidal and anti-virucidal features against invasive pathogens and its ability to increase plant immunity. Numerous research on the mechanism by which biotic stress induces chitosan-induced resistance have been thoroughly discussed previously (Katiyar et al., 2014, Pichyangkura and Chadchawan 2015, Sharif et al., 2018). Plants have been able to withstand abiotic conditions such high temperature stress, salt, water deficiency, and toxic effects of heavy metals by using chitosan and its oligomers (Malerba and Cerana 2015). Experts are interested in exploring this unique biopolymer further and offering a wider range of applications due to its ability to scavenge ROS systems and eventually increase efficiency during stress. Chitosan application enhances the activity of photosynthesis (Li et al., 2008), strengthens the capacity of water uptake by improved root growth (Zeng and Luo 2012), and mitigates the negative effects of antimicrobial enzymes in drought stress (Yin et al., 2008). This review compiles the latest information on chitosan sources, optimal concentrations for effective application under various types of stresses in different crops, and the mechanisms through which it acts to manage both biotic and abiotic stresses, ultimately enhancing crop production.

## 2. Sources of Chitosan

According to Pornpienpakdee et al. (2010), chitosan is a most important naturally occurring biopolymer that is extracted from deacetylation of chitin, a vital structural polysaccharide that makes up a significant portion of the exoskeletons of insects and crustaceans. The various sources of chitosan are the aquatic (crustaceans), terrestrial (arthropods), and microorganisms like fungi, blastomycota, Chytridiomycota, protist, planta. From crustaceans (crabs, water lobster, prawn, krill, mollusca, coelenterate and from arthropods (spiders, scorpionxs, beetles, cockroaches, brachiopods) are the different sources for chitosan (Table 1).

## 3. Chemical Structure and Production of Chitosan from Chitin

Table 1: Various sources of chitin and chitosan

Source	Crustaceans	References
Aquatic	Crab	
	<i>Chionoecetes opilio</i> ,	Crespo et al. (2006)
	<i>Podophthalmus vigil</i> ,	Das et al. (2010)
	<i>Paralithodes</i> ,	Sperstad et al. (2009)
	<i>Carcinus</i>	Hajji et al. (2014)
	<i>mediterraneus</i>	
	Water lobster	
	Cray fish	Abdou et al. (2008)
	Prawn	
	<i>Aristens antennatus</i>	Mahlous et al. (2007)
Terrestrial	Krill,	
	<i>Daphnia longispina</i> ,	
	<i>Anax imperator</i> ,	
	<i>Hydrophilus piceus</i> ,	Kaya et al. (2014)
	<i>Notonecta glauca</i> ,	
	<i>Agabus bipustulatus</i> ,	
	<i>Asellus aquaticus</i>	
	Mollusca	
	<i>Loligo sp</i> ,	Chaussard et al. (2004)
	<i>Todarodes pacificus</i>	Fan et al. (2008)
	coelenterata	
	Arthropods	
	Spiders	
	<i>Geolycosa vultuosa</i> ,	Kaya et al. (2014)
	<i>Hogna radiate</i> ,	
	<i>Nephila edulis</i>	Davies et al. (2013)
	<i>Scorpionxs</i>	
	<i>Mesobutus gibbosus</i>	Kaya et al. (2016)
	Beetles	
	<i>Bombyx mori</i> ,	
	<i>Holotrichia parallela</i> ,	Zhang et al. (2000)
	<i>Leptinotarsa</i>	Liu et al. (2011)
	<i>decemlineata</i>	Zhang et al. (2000)
	Cockroaches	Kaya et al. (2015)
	Brachiopods	
	<i>Lingula seta</i>	Tanaka et al. (1988)
Fungi	Mushroom	Islam et al. (2017)

### 3.1. Chemical structure of chitosan

According to Dutta et al. (2004), chitosan is a co-polymer of N-Acetyl Glucose Amine (2- Acetamido- 2- Deoxy- - D- Glucose) and glucosamine (2- Amino- 2- Deoxy- - D- Glucose). Chitosan's mechanical and physical properties are influenced by reactive functional groups, including the amino group (C<sub>2</sub>) and 1° & 2° hydroxyl groups (C<sub>3</sub> & C<sub>6</sub>). Shahidi et al., 1999 reported that these groups contribute to the material's flexibility in a variety of applications (Figure 1). The biological



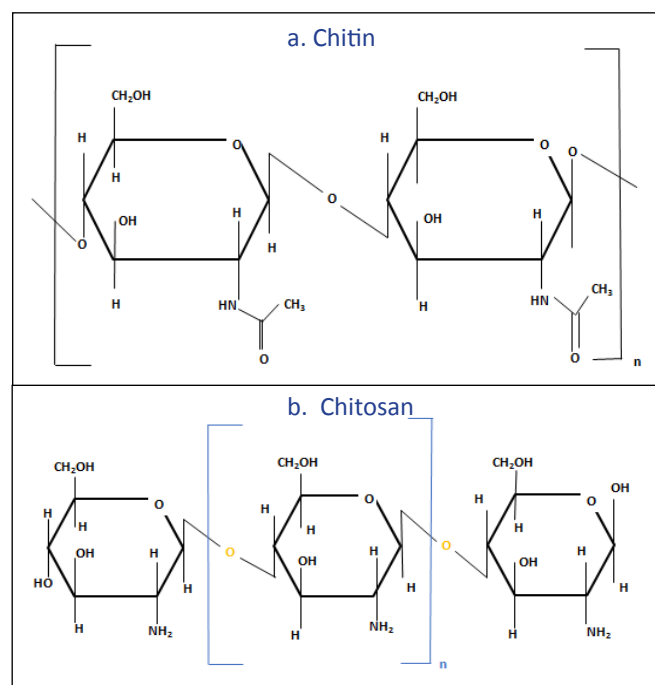


Figure 1: Structure of chitin and chitosan

applications of chitosan are reported in agriculture, food technology, biotechnology, medicine and pharmacy and environmental protection (Pongprayoon et al., 2022). In agriculture chitosan treated on numerous plant species, including cereal crops, pulses, oil seed crops, cash crops, aromatic, ornamental crops, horticultural crops and medicinal crops. In plants chitosan effects depend on the structure and amount, type of plant, and critical growth stages of the plant (Ohta et al., 2004; Pornpienpakdee et al., 2010).

### 3.2. Production of chitosan from chitin

Figure 2 illustrates how to prepare chitosan from chitin. According to Islam et al. (2017), chitosan is made by hydrolyzing the acetamido reactive functional groups ( $-\text{NHCOCH}_3$ ) of chitin found in fungi and crustaceans (prawn, shrimp and crab). But chitosan is industrially prepared from chitin by processes including decolorization, deacetylation, demineralization, and deproteinization. According to Aranaz et al. (2009), the typical composition of a crustacean shell is 20%–30% pigments called carotenoids, 30%–40% proteins, and 30%–50%  $\text{CaCO}_3$  (calcium carbonate). Usually, sodium hydroxide (NaOH) is used for the deacetylation stage, which produces 70% deacetylated chitosan when heated to  $120^\circ\text{C}$  for one to three hours (Dutta et al., 2004). Chitosan is produced when incomplete deacetylated chitin (less than 30%) is exposed to an alkaline solution at an amount of 30%–50% (w/v) at  $100^\circ\text{C}$  (Aranaz et al., 2009).

## 4. Mechanism of Chitosan Against Plant Biotic and Abiotic Plant Resistance

The exact mechanism of chitosan in crops is still unknown. But according to Mejia-Teniente et al., 2013, Malerba and Cerana

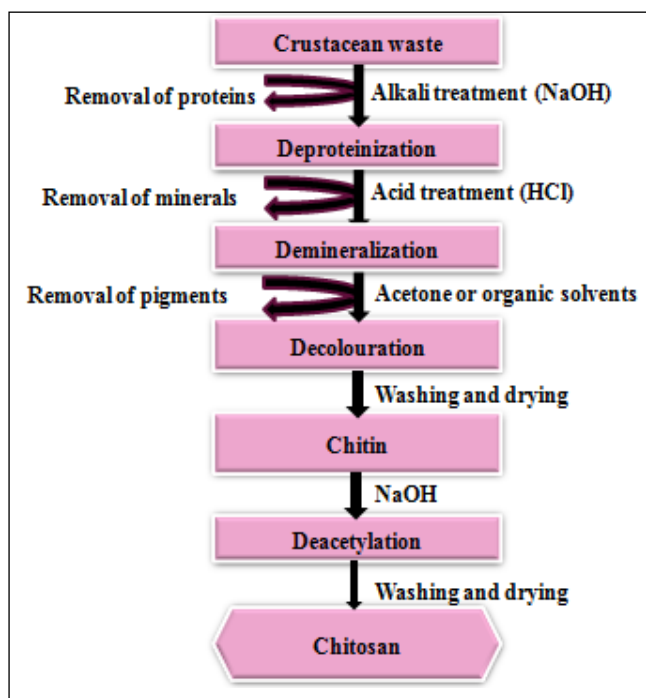


Figure 2: Steps for production of chitosan from chitin

2015 reports data indicate that chitosan triggered various defence reactions in crops. Iriti and Faoro, 2009 reported that plant cell membranes have receptors that are specific to chitin and are known to trigger immune reactions. Plants that receive chitin-based treatments trigger their defence mechanisms because the molecules they resemble are those found in chitin-containing organisms. Chitosan also developed defence mechanisms in response to biotic stress, which included the creation of anti-microbial compounds like phytoalexins, biosynthesis of lignin, PR-proteins (pathogenesis related- proteins) like-glucanases and chitinases, different proteinase inhibitors, callose formation, and the stimulation of SR-genes (stress responsive-genes). Chitosan and its derivatives can be used as potent antibacterial chemicals and elicitors for plant protection since they boost defence-related molecules when treated with chitosan and its oligomers (Katiyar et al., 2014).

### 4.1. Signal transduction of chitosan in plants

Meena et al., 2022 reported that the modification of the cell membrane's ion permeability is a typical defence signalling tactic. Pongprayoon et al., 2022 also reported that chitosan interacts with plant cells by binding to certain receptors, which causes secondary messengers including  $\text{H}_2\text{O}_2$ , calcium ion ( $\text{Ca}^{2+}$ ), NO, and phytohormones to be released inside the cell and cause physiological reactions. The mechanism and signal transduction of chitosan in plants is presented in Figure 3. In rice plant  $\text{H}_2\text{O}_2$  acts as signal molecule under osmotic stress it preserving photosynthetic pigments and stimulating plant development. By controlling the activity of callose synthase, chitosan stimulates the production of  $\text{Ca}^{2+}$  in plant species, which causes soyabean cells to undergo

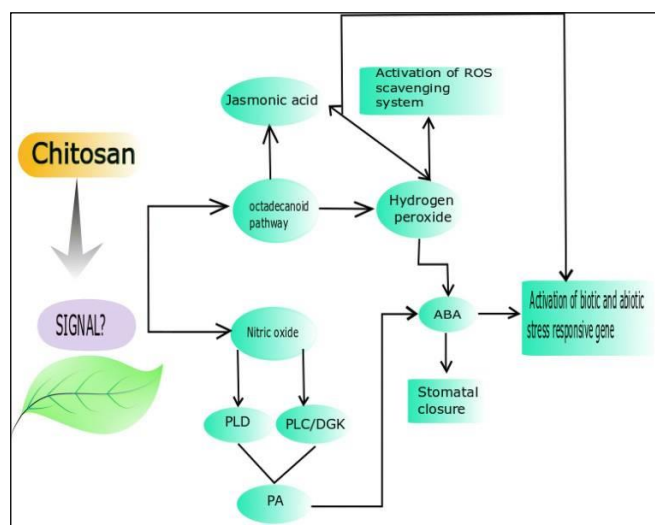


Figure 3: Role of chitosan mediated responses in stomata closing mechanism via ABA biosynthesis at cellular level. The signaling induced by chitosan involves hydrogen peroxide via octadecanoid pathway and nitric oxide. Hydrogen peroxide induces both the ABA synthesis and ROS scavenging mechanism while nitric oxide regulates phosphatidic acid (PA) via phospholipase C (PLC) and diacylglycerol kinase (DGK) (PLC/DGK) pathway, and initiates ABA synthesis leading to closure of stomata and activates biotic and abiotic stress responsive genes (Figure courtesy of Pichyangkura and Chadchawan 2015).

Ca<sup>2+</sup>-mediated programmed cell death. However, chitosan-treated pearl millet seedlings have been discovered to have

NO-signaling. In leaf tissues, chitosan treatment led to a buildup of ABA and induced resistance to the tobacco necrosis virus (TNV). Jasmonic acid (JA) accumulates in a number of plants, including tomato, French bean, and rapeseed, as a result of chitosan.

Salicylic acid (SA) and jasmonic acid (JA) are two important plant hormones which play crucial role in signal transduction that promotes disease and pest resistance in plants. SAR (systemic acquired resistance) is regulated by JA, whereas SA regulates systemic induced resistance. Chitosan treatment could promote oxidative production, nitric oxide (NO) in the chloroplast, activation of MAP kinases, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) through octadecanoid pathway, and hypersensitivity reactions. These signal molecules influence the plant's ability to adjust to biotic and abiotic challenges in chitosan-treated plants.

### 5. Application of Chitosan to Combat Biotic Stress

There is a greater chance of hunger worldwide as a result of biotic stress-induced agricultural product deterioration. Plants utilise a variety of biochemical, morphological, and molecular strategies to combat these stressors. Chitosan treated crops can trigger defence reactions in response to biotic stress, such as the production of antimicrobial compounds (phytoalexins), proteinase inhibitors, chitinases and -glucanases (PR- proteins) (Pichyangkura and Chadchawan, 2015). It is clearly shown in Table 2. The chitosan oligomers have been shown to enhance defence-related substances and act as antibacterial compounds, which in turn elicits plant defence (Katiyar et al.,

Table 2: Protective effect of chitosan against biotic stress

Crop	Concentration and method of chitosan application	Mode of action/protective effects	References
<i>Solanum lycopersicum</i>	Fruit dipping in postharvest	Induced production of rishitin (a phytoalexin)	Reddy et al. (2000)
<i>Capsicum annuum</i> L.	1% chitosan, foliar application	Resistance against <i>Phytophthora capsica</i>	Esyanti et al. (2019)
<i>Melissa officinalis</i>	0.005, 0.01, 0.015% chitosan, shoot spraying	Accumulation of defence-related enzymes and phenolic compounds.	Vanda et al. (2019)
<i>Vitis vinifera</i> L.	Excised leaf incubation	Increased glucanase activity	Trotel-Aziz et al. (2006)
<i>Selenicereus undatus</i>	Fruit dipping in post-harvest	Increased glucanase and chitinase activities	Ali et al. (2014)
<i>Solanum lycopersicum</i>	0.001, 0.01, 0.1% chitosan microparticles, foliar application	Accumulation of defence-related enzymes	Colman et al. (2019)
<i>Oryza sativa</i> L.	0.3% chitosan oligosaccharide, seedlings spraying	Resistance against <i>Fusarium oxysporum</i>	Ma et al. (2019)
<i>Solanum tuberosum</i> L.	0.4% chitosan, tuber immersion	Resistance against <i>Fusarium</i> spp.	Mejdoub-Trabelsi et al. (2020)
<i>Pisum sativum</i> L.	Application on the surface of pea pods	Induced proteinase inhibitor (pisatin)	Walker-Simmons et al. (1983)



2014). Tomato fruit dipping in chitosan induces production of rishitin (a phytoalexin) resistance against postharvest disease of tomatoes (blackmold) caused by pathogenic fungi (*Alternaria alternata*) (Reddy et al. (2000)). Chitosan foliar application improves growth and alters the regulation of defence genes in pepper plant (*Capsicum annuum*), thus decreasing the substantial yield losses in chilli production infection caused by phytophthora species (*Phytophthora capsici*) (Esyanti et al., 2019). Vanda et al., 2019 found that a number of phenolic compounds and defence related enzymes with anti-microbial properties are accumulated in perennial herb lemon balm (*Melissa officinalis*) shoot cultures treated with chitosan.

Application of chitosan induces pathogenesis related compounds (glucanase and chitinase) in grape and dragon fruits. Likewise, Mejdoub-Trabelsi et al., 2020 in potato (*Solanum tuberosum*), Ma et al., 2019 in rice (*Oryza sativa*) reported that chitosan mitigates diseases induced by fusarium species. According to Iwasaki et al., (2020), suppressive properties of chitin added to the soil in opposition to pathogenic microbes frequently entails a modification in the composition of the microbial community in the soil with an increase in the presence and activity of chitinolytic microbes that hydrolysis the chitinous hyphae of the pathogenic fungi, and with an increasing number of 2° responders to added chitin that might affect pathogens. However, it has been noted that soil-borne fungal infections primarily employ the deacetylation of chitin oligomers by certain enzymes, turning them into ligand-inactive chitosan, as a means of circumventing the protective function of chitin (Gao et al., 2019).

### 5.1. Effect of chitosan on fungi, bacteria, viruses, nematodes and insects

Allan and Hadwiger first described chitosan as a bio-fungicide in 1979, since it has received a lot of attention from scientists studying plant protection. Its effectiveness in preventing bacterial-caused plant disease is also extensively recognised. The most workable method of reducing viral infection was to use chitosan as a virucide. According to research by Chirkov et al. (2001), potato virus X (PVX) plants are treated with chitosan shows resistant against PVX virus. Furthermore, Application of chitosan on tomato plants demonstrated enhanced vegetative development in addition to resistance to tomato mosaic virus. Oligomeric chitosan increases resistant against tobacco mosaic virus (TMV) by stimulation of the salicylic acid signalling system.

Application of chitosan inhibits the growth and development of numerous fungal pathogens like *Botrytis cinerea*, *Sphaerotheca fuliginea* in cucumber (Ben-Shalom et al., 2003, Moret et al., 2009), *Sclerotinia sclerotiorum* in carrots (Cheah et al., 1997), *Colletotrichum capsici* in chilli pepper (Long et al., 2018), *Colletotrichum gloeosporioides* in mango (Jitareerat et al., 2007), *Penicillium italicum* and *Penicillium*

*digitatum* in orange (Zeng et al., 2010), *Alternaria kikuchiana* and *Physalospora piricola* in pear (Meng et al., 2010), *Fusarium oxysporum* in palm (Bautista- Banos et al., 2003), *Monilinia fructicola* in peach (Ma et al., 2013), *Anthraco* in banana (Jinasena et al., 2011). Its antifungal action's underlying mechanism is still being investigated. Nonetheless, two theories have been put up to account for chitosan's antifungal properties. According to Leuba and Stossel's (1986) theory, chitosan's activity is correlated with its capacity to obstruct the function of the plasma membrane. According to Hadwiger and Loschke (1981), chitosan's antifungal effect may be attributed to its interactions with fungal DNA and mRNA. It appears that there could be multiple mechanisms involved in chitosan impact on inhibition.

To evaluate the role of chitosan as a potential nematicide, more research is necessary because there isn't a lot of information about nematodes. Root-knot nematodes, are the most harmful pests for horticulture crops globally. Bio-agents may offer a non-chemical approach for managing these nematodes. The nematophagous fungus *Pochonia chlamydosporia* is a parasite of root-knot nematode eggs. It may colonise the roots of various cultivated plant species endophytically, however in field applications, it exhibits poor persistence and efficacy in managing nematodes. When *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) is used in conjunction with an enhancer, it may be able to grow in soil and colonise roots more easily, which would increase its effectiveness against nematodes (Escudero et al., 2017). Numerous research studies have examined the efficiency of chitosan against various infections and pests in relation to various fruits and vegetables, it is enlisted in (Table 3). So, from the vast body of chitosan research, we selected current publications describing chitosan's ability to be fungicidal, bactericidal, virucide and nematocidal in agriculture. The body of research points to the potential use of chitosan as a bio-stimulant to combat various hostile situations.

The most important active derivative of chitin is (N-2-CHLORO-6-FLUOROBENZYL) chitosan, was found 100% fatal to aphids and leafworms in cotton crops (Rabea et al., 2005). Sahab et al. (2015) also report that chitosan nanoparticles (CS-g-poly acrylic acid) has been identified as a potential bioinsecticide against cotton & melon aphids (*Aphis gossypii*) and cowpea seed beetle (*Callosobruchus maculatus*), two insects connected to soybeans, as it considerably lowers the quantity of eggs that produced by female. Lately, Avermectin (AVM)-grafted NOCC, a novel derivative of chitosan, was discovered and proven to have outstanding insecticidal properties against species frugiperda (army worms), brown plant hoppers, blackfly (black bean aphids), carmine spider mites (Li et al., 2016). It is advantageous to use chitosan as a bioinsecticide on horticultural and agricultural crops.

### 6. Application of Chitosan Resistance to Abiotic Stress

According to Ben-Ari et al., 2012, Detrimental effects of abiotic



Table 3: Effect of chitosan against different pathogens

Crop	Concentration/method of application	Effect of chitosan against pathogen	Reference
Cucumber	0.2 g l <sup>-1</sup> chitosan+1.6 millimole copper, Foliar spray	<i>Botrytis cinerea</i> Antifungal	Ben-Shalom et al. (2003)
Carrots	2 or 4% (w/v), <i>in vitro</i>	<i>Sclerotinia sclerotiorum</i> Antifungal activity	Cheah et al. (1997)
Cucumber	2% (w/v), Petri dish treatment	<i>Sphaerotheca fuliginea</i> Antifungal	Moret et al. (2009)
Chilli pepper	0.32% (w/v), <i>in vitro</i>	<i>Colletotrichum capsici</i> Hijacked fungal activity	Long et al. (2018)
Mango	1% (w/v) Post-harvest coating	<i>Colletotrichum gloeosporioides</i> Fungus inhibition	Jitareerat et al. (2007)
Orange	2% (w/v) Post-harvest coating	<i>Penicillium italicum</i> and <i>Penicillium digitatum</i> Fungicidal effect	Zeng et al. (2010)
Pear	25 g l <sup>-1</sup> Post-harvest treatment	<i>Alternaria kikuchiana</i> and <i>Physalospora piricola</i> Antifungal activity	Meng et al. (2010)
Palm	1 mg ml <sup>-1</sup> soil inoculation	<i>Fusarium oxysporum</i> Inhibition of root fungal activity	Bautista-Banos et al. (2003)
Peach	0.5 g l <sup>-1</sup> Dipping in solution	<i>Monilinia fructicola</i> Antioxidant and antifungal	Ma et al. (2013)
Banana	1.0% (w/v) chitosan, <i>in vivo</i>	<i>Anthraco</i> Arresting fungal activity	Jinasena et al. (2011)
Tomato	0.1 mg ml <sup>-1</sup> Fertigation	Root-knot nematode Nematocidal effect	Escudero et al. (2017)

factors on biotic organisms in a specified ecosystem is simply known as abiotic stress. Abiotic factors such as drought, high salinity, heat, heavy metal stress, which shows negative impact on crops it results worldwide reduction in crop production and productivity (Zaidi et al., 2014). To combat these type of stresses chitin & chitosan are the compounds that have been examined & have the potential ability to increase tolerance to a variety of abiotic stress. The overview of effect chitosan on abiotic stress is presented in Figure 4.

#### 6.1. Effect of chitosan on drought stress

Reduced agricultural production due to drought stress or inadequate irrigation results in various detrimental effects on crop health. These primarily involve the generation of byproducts like reactive oxygen species (ROS). They can cause lipid-peroxidation in membrane and interact with various other macromolecules, ultimately it leads to decreased yield, growth and development of the plant (Yang et al., 2009; Bistgani et al., 2017). However, chitosan applications promoted plant growth and improved water and nutrient uptake, which in turn improved scavenging activities (Guan et al., 2009).

Chitosan improves the plant's tolerance to drought stress by modifying its metabolic processes. Proline concentration increased in drought stress. Proline is an essential osmo-

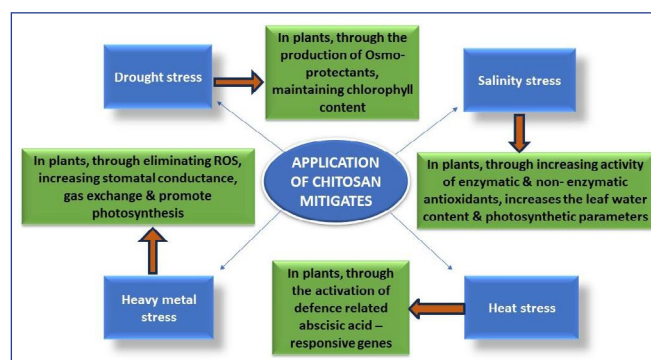


Figure 4: Mechanism of action of chitosan to combat abiotic stresses in plants

protectant that helps maintain redox balance in the face of abiotic stresses that alter osmotic pressure, and quench reactive oxygen species (Ashraf and Foolad, 2007; Hidangmayum, 2019). As part of an adaptation strategy, metabolic factor including the free proline content in leaves drastically increased under extreme drought stress (Din et al., 2011). Leaf water potential is lowered by proline accumulation, which helps to reduce water loss. Additionally, it promotes leaf water transfer and raises leaf turgor. According to research by Joshi et al. (2010), Chang et al. (2014), Du et al., (2016), Singh and Dwivedi (2016), Amino

acids including - amino acid (aspartic acid, isoleucine), lysine, and L-threonine have also been found as osmo-regulators, providing nourishment when plants experience adverse effects (abiotic and biotic stresses).

Because of the production of free radicals in a low-water environment, MDA levels increase and can lead to membrane leakage. Lipid peroxidation produces MDA as a byproduct. Chitosan, on the other hand, decreases the damaging effects of drought stress symptoms by acting as a beneficial factor in osmotic adjustment. Numerous studies have shown that pretreating potato, thyme, bean and apple seedlings with chitosan reduces peroxidation of lipids, eliminates reactive oxygen species, and improves membrane integrity (Jiao et al., 2012, Bistgani et al., 2017). Because chitosan contains a large number of amine groups ( $\text{NH}_2$ ) and hydroxyl groups, when these combine with ROS, they create stable, non-toxic macromolecular radicals. Chitosan has DNA-protective qualities and the ability to scavenge OH and  $\text{O}_2$ -radicals (Prashanth et al., 2007). Apple seedlings are treated with chitosan it results increased superoxide dismutase (SOD) and catalase activity encourage the synthesis of malondialdehyde (MDA) and lessen peroxidation of lipids under drought stress (Yang et al., 2009). According to sun et al., 2004, Superoxide anion can be protected by chitosan. According to Liu et al. (2011), soluble sugars help root crops like sugar beets, pulses, and lombardy poplar (black poplars) withstand drought. Sugars like fructose and glucose help plants withstand drought by regulating their physiology & stress responses through mechanism of signal transduction pathway (Rolland et al., 2006). Sugars (carbohydrates) such as dextrose(glucose), semiose (mannose), fruit sugar(fructose), D-glucitol(sorbitol), mycose (trehalose) are increased in chitosan treated vegetation, whereas other genes related to the metabolism and transportation carbohydrates were also up-regulated by additional sugars found in perennial plant white clover (*Trifolium repens*) leaves (Li et al., 2017). Likewise, Foliar application of chitosan on wheat plants results increased osmolytes production such as proline, sugars like mannitol, glucitol, and mycose (Farouk et al., 2019). Oxidative stress tolerance can be achieved in seed treatment of chitosan in safflower under drought stress (Mahdavi et al., 2011). On the other hand, when chitosan is sprayed at a concentration of  $250 \text{ mg l}^{-1}$  on lobiya (*Vigna unguiculata*), it was shown that the effect on total carbohydrate and chlorophyll content ultimately it leads to increased plant growth and yield (Farouk and Amany, 2012). Chitosan effect on drought stress in different crops is enlisted in (Table 4).

According to Sheikha and Al-Malki (2011), Khan et al. (2002), treating maize, soybean, and beans with chitin oligosaccharides resulted in a similar rise in photosynthesis levels. This could be the result of higher levels of potassium and nitrogen in plant shoots, which raise the total number of chloroplasts with in cell and thus boost synthesis of chlorophyll (Possingham, 1980). Gornik et al. (2008) reported

that chitosan treated grape stem cuttings can be maintained chlorophyll content under drought stress conditions. Application of chitosan on various crops rice (Boonlertnirun et al., 2007), wheat (Zeng and Luo, 2012), basil (Malekpoor et al., 2016) alleviate drought stress by different mechanisms. In rice by inducing hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) production, whereas wheat increased antioxidants and by improving chlorophyll content. Chlorophyll production is also stimulated by the increased amount of amino acid compounds produced by chitosan application (Chibu and Shibayama, 2001). Under drought conditions, an increase in proline production results reduction in the synthesis of photosynthetic pigments due to proline and photosynthetic complexes. (Paleg and Aspinall, 1981). Absciscic acid is produced during conditions of drought stress, it act as a signaling molecule and it induces the stomatal closure. This might lower the level of carbon dioxide ( $\text{CO}_2$ ) inside cells, which would lower the rate of photosynthesis reactions. Researchers refer to this as stomatal limitation. In the alternative pathway, a drought will lower the plant water status (relative water content) which may lower the activity of the Rubisco enzyme and decrease the  $\text{CO}_2$  fixation. It is known as non-stomatal limitation. Stomatal and non-stomatal limitation can be reduced by foliar application 0.01% chitosan on Maize plants (Veroneze-Junior et al., 2019). oil and protein content abundant in seeds of mustard, drought stress decreases the oil quality in mustard seeds. Mustard seedlings are soaked in 0.2% chitosan solution results decreases the negative effects of different fatty acids it leads to improved oil quality (Noormohamadia et al., 2019).

## 6.2. Effect of chitosan on salt stress

According to Hidangmayum et al. (2019), salinity stress causes a negative impact on plant physiological processes and biochemical mechanisms. In extreme situations, this stress can even prevent plants from taking water and nutrients. This is caused by less external solute potential that result in a greater accumulation of sodium & chloride ions. Oxidative stress is caused by salt stress, which generates free radicles (reactive action species) and modifies biochemical processes that disrupt transcription, DNA replication etc (cellular processes). In salinity stress conditions increased malondialdehyde concentration was reported, as a result of ion toxicity-induced membrane lipid peroxidation, as other investigations have found. Still, several researchers have proposed low-concentration chitosan treatment as a means of mitigating the deleterious consequences of salinity stress (Hidangmayum et al., 2019). Low concentration chitosan-treated seeds of sunflower & safflower can lessen the effects of salinity stress, since they both show decreased enzyme activity. (Jabeen and Ahmad, 2013). CH pretreatment during salt stress results in raised activity of antioxidant enzymes and a decreased amount of malondialdehyde, just as in the cases of green gram (Ray et al., 2016), ajwain (Mahdavi and Rahimi, 2013), maize (Al-Tawaha et al., 2018), desert indianwheat (Mahdavi, 2013) and rice (Martinez et al., 2015).



Table 4: Effect of Chitosan on drought stress in various crops

Plant	Method of application	Mode of action/Effects	Reference
Apple ( <i>Malus sieversii</i> )	Foliar applied (100 mg l <sup>-1</sup> )	Enhanced leaf membrane stability, increased antioxidant enzymes (superoxide dismutase and catalase activity)	Yang et al. (2009)
White clover ( <i>Trifolium repens</i> )	1 mg ml <sup>-1</sup> ,	Under drought stress accumulation of stress protective metabolites	Li et al. (2017)
Wheat ( <i>Triticum aestivum</i> L.)	0.0125% chitosan, foliar application	Drought stress achieved through enhanced the accumulation of osmolytes	Farouk et al. (2019)
Safflower ( <i>Carthamus tinctorius</i> L.)	Seed treatment (0.05–0.4%),	Decreased enzyme activity and increased oxidative stress tolerance and seedling growth	Mahdavi et al. (2011)
Cowpea ( <i>Vigna unguiculata</i> L.)	Foliar applied (250 mg l <sup>-1</sup> ).	Improved growth and yield	Farouk and Amany (2012)
Grape vine	Dipping of stem cuttings before planting.	Drought stress achieved through maintaining chlorophyll content	Gornik et al. (2008)
Rice ( <i>Oryza sativa</i> )	Seed soaking and foliar application on seedlings, effect is induced H <sub>2</sub> O <sub>2</sub> production	Drought stress achieved through induced H <sub>2</sub> O <sub>2</sub> production	Boonlertnirun et al. (2007)
Wheat ( <i>Triticum aestivum</i> L.)	Seed treatment.	Drought stress achieved through improved chlorophyll content, and antioxidant enzymes	Zeng and Luo (2012)
Basil ( <i>Ocimum basilicum</i> L.)	Foliar applied (0.4 g l <sup>-1</sup> ).	Enhanced plant growth	Malekpoor et al. (2016)
Maize ( <i>Zea mays</i> L.)	0.01% chitosan, foliar application	Drought stress achieved by decreasing stomatal and non- stomatal limitation	Veroneze-Junior et al. (2019)
Mustard ( <i>Brassica napus</i> L.)	0.2% chitosan, seedling soaking	Drought stress effects on oil quality in mustard. Chitosan application reduced the harmful effect of this two fattyacids linolenic and erucic acids, it leads to increase in oil quality	Noormohamadia et al. (2019)

Effect of chitosan mitigates the salinity stress in numerous crops enlisted in (Table 5). Finally, this lessens the detrimental effects of salt stress. Ma et al., 2012 conducted a hydroponic study conducted on wheat also revealed that percentage of 0.0625 chitosan-oligosaccharide treated seed had favourable outcomes by markedly raising superoxide dismutase and catalase enzymes concentration during induced salinity stress and was able to reduce oxidative stress.

Through the synthesis of enzymatic antioxidants (SOD, CAT and GPX), nonenzymatic antioxidants eliminates a number of free radicals (ROS) in plants. According to Jabeen and Ahmad (2013), plants that are exposed to salt stress have higher levels of the enzyme antioxidants SOD, POD, and CAT. An effective ROS detoxification is indicated by a higher concentration of these enzymes. Figure 5 presents an overview of effects of salinity stress sensitive plants and salinity stress tolerant

plants (effect of chitosan on salinity stress plants). According to studies conducted by Ma et al. (2012), Jabeen and Ahmad (2013), plants treated with chitosan exhibit an increase in these enzymes and have a significant effect on reducing salt stress by enhancing antioxidant enzymes. Similarly, in salinity stress conditions, peroxidation of lipids due to malondialdehyde (MDA) buildup was discovered (Meloni et al., 2003). Jabeen and Ahmad (2013) found that treatment with chitosan resulted in decreased MDA level, it finally stabilizes membrane damage which may be the cause of conferring tolerance against salinity stress. Fenugreek seeds are treated with CH increases the leaf water content and photosynthetic parameters results salinity stress tolerance (Yahyaabadi et al., 2016). Chanratana et al. (2019) reported that CH used as a bio-inoculant in tomato cultivation because it supplies nutrients to plants, and improves growth activity of





Table 5: Effect of chitosan on salinity stress in various crops

Crop	Mode of application	Mode of action/Effect	References
Sunflower ( <i>Helianthus annuus</i> )/Safflower ( <i>Carthamus tinctorius</i> )	Seed treatment (0.25%),	Increased germination percentage, alleviates salt stress through reduction of enzyme activity on both crops	Jabeen and Ahmad (2013)
Mung bean ( <i>Vigna radiata</i> )	Seed treatment	It Stimulate the morphological parameters and alleviate salt stress	Ray et al. (2016)
Ajowan ( <i>Carum copticum</i> )	Seed treated with 0.2% chitosan	Increased shoot and root length and adjusted salt toxicity	Mahdavi and Rahimi (2013)
Maize ( <i>Zea mays</i> )	Foliar application	Enhanced all the growth parameters and alleviated salt stress	Al-Tawaha et al. (2018)
Rice ( <i>Oryza sativa</i> )	Seed treatment	Salt stress achieved through enhanced catalase and peroxidase enzymes.	Martinez et al. (2015)
Wheat ( <i>Triticum aestivum</i> L.)	0.0625% oligochitosan added to nutrient solution	Alleviated adverse effect of salt stress	Ma et al. (2012)
Tomato ( <i>Solanum lycopersicum</i> )	Application of chitosan–aggregated growth-promoting bacteria	Under salt stress conditions, Chitosan used as a bioinoculant for plant growth	Chanratana et al. (2019)
Maize ( <i>Zea mays</i> cv. Arififiye)	0.1% chitosan, foliar application	Chitosan mitigating effect on salt stress is linked to activation of alternative respiration at biochemical and molecular level	Turk (2019)
Fenugreek ( <i>Trigonella foenum graecum</i> L.)	Seed treatment with 1 g l <sup>-1</sup>	Improved leaf water content, photosynthetic parameters and alleviated salt stress	Yahyaabadi et al. (2016)

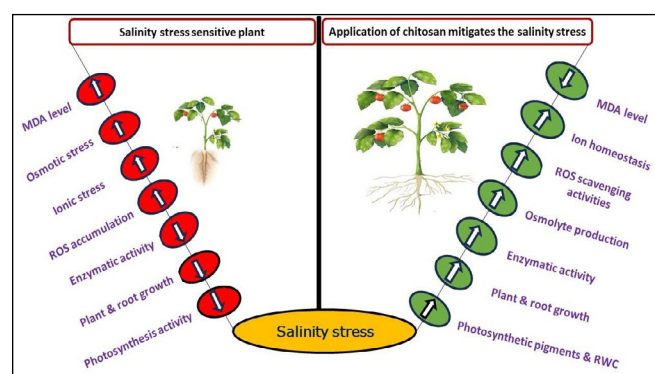


Figure 5: Mitigative effects of chitosan in salinity stressed plants

plant in salinity stress conditions. Foliar application of CH at the rate of 0.1% in maize plants alleviates the salinity stress through activation of alternative respiration (Turk, 2019).

### 6.3. Effect of chitosan on heavy metal toxicity

Cellular disfunction and metabolism are brought on by heavy metal toxicity in shoots and roots under heavy metal stress. Chitosan may develop complexes with a variety of toxic metals because it has functional amino and hydroxyl groups. Additionally, chitosan has several functional groups, such as an aminogroups (NH<sub>2</sub>) and hydroxyl group (OH), which play the roles of adsorbing various heavy metals and reducing their uptake by plants (Zubair et al., 2021). Impact of chitosan on heavy metal stress in various crops listed in

(Table 6). In addition to these uses, chitosan raises the pH of soil, which increases the amount of negative charges present and encourages the development of hydroxyl-bound HM species (HMsOH<sup>+</sup>), which in turn promote metal precipitation. These procedures lessen the soil's bioaccessibility to heavy metals and the plants' ability to absorb them (Zubair et al., 2021). Maize seedlings are treated with chitosan at the rate of 0.01%, it achieved cadmium stress tolerance through decreasing reactive oxygen species accumulation, antioxidants breakdown and increased amount of chlorophyll (Qu et al., 2019)

The detrimental effects of cadmium in field mustard (*Brassica rapa*) grown hydroponically can be lessened by applying chitosan with different molecular weights by foliar spray, according to recent research (Zong et al., 2017a). According to reports, chitosan applied externally tends to mitigate the effects of cadmium (Cd) toxicity and improve photosynthesis, transpiration (Zong et al., 2017a). Cd toxicity is known to reduce physiological process. However, it was shown that ascorbic acid decreased in response to Cd stress. This finding may indicate that ascorbic acid functions as the body's first line of defense against oxidative stressors due to its potential mechanism to directly eliminate the free radicles. According to Zong et al. (2017a), whereas chitosan treatment greatly raised the amount of ascorbic acid in Cd stress plants, glutathione levels were found to rise under Cd stress but to stay stable with chitosan treatment. This demonstrates that glutathione

Table 6: Effect of chitosan on heavy metal stress in various crops

Plant	Stress	Method of application	Mode of action/Effect	References
Edible rape ( <i>Brassica rapa</i> L.)	Cadmium stress (cd)	Applying chitosan foliarly (10kDa, 5kDa, or 1kDa in molecular weight)	Reported lower levels of Cd in edible rapeseed shoots.	Zong et al. (2017a, b)
Aubergine	Heavy metal stress (Ni, Cd, Co, Cr, Pb.)	Soil amendment	Chitosan has a beneficial effect on plant growth and increases the amount of protein, fat, fibre, and carbohydrates in aubergine.	Turan et al. (2018)
Maize ( <i>Zea mays</i> L.)	Cadmium stress tolerance	0.01% chitosan, seedling soaking	Increased the amount of chlorophyll and enhanced plant photosynthesis by reducing the breakdown of antioxidant enzymes and preventing the generation of reactive oxygen species.	Qu et al. (2019)
Moringa ( <i>Moringa oleifera</i> Lam.)	Cadmium stress (cd)	Soil amendment	It significantly increase the flavonoids, protein, lipids, alkaloids, and tannins in moringa plant.	Zubair et al. (2021)

is inert when exposed to chitosan. Likewise, in an identical experiment conducted in a greenhouse, cadmium (Cd) toxicity was found to have a protective effect (Zong et al., 2017b; Turan et al., 2018). After applying chitosan to soil contaminated with heavy metals like lead(pb), cadmium(cd), chromium (cr), nickle (Ni), and cobalt(co), the researchers discovered a notable increase in the amount of carbohydrates, dietary fats, total soluble protein, and fibre in eggplant. In a different study, adding CH to soil contaminated with Cd greatly increased the amount of secondary metabolites such as tannins, flavonoids and alkaloids i.e; amines, total soluble protein, lipids in the moringa trees (Zubair et al., 2021).

#### 6.4. Effect of chitosan on heat stress

There is not much published research on using chitosan when under heat stress. Because it typically coexists with drought stress and is difficult to determine, heat stress is commonly viewed as a complicated issue (According to McKersie and Lesheim, 2013). Ibrahim and Ramadan (2015) have discovered that late-sown beans may be able to withstand heat stress

when chitosan is sprayed on them along with zinc and humic acid. According to Choi et al. (2013), abscisic acid has been shown to activate genes linked to heat stress. Hussain et al. (2019) reported that under plastic tunnel conditions foliar application of chitosan at different concentrations (0.006, 0.012, 0.003, 0.003%) mitigate heat stress in tomato seedlings through improving growth and quality parameters. Chitosan application mitigates the heat stress in tomato (Hussain et al., 2019), Cayenne pepper plant (Al- Hassani and Majid, 2019), and Egg plant (Liaqat et al., 2019) are briefly listed in (Table 7). One potential solution to improve heat stress tolerance is ABF3 or Absciscic acid responsive-element-binding factor 3 (Hidangmayum et al., 2019). Hence, by promoting ABA activity-which is connected to the earlier study on closing of stomata (Bittelli et al., 2001) and further promoting defence-related abscisic acid-responsive genes, the chitosan treatment could potentially mitigate the negative effects of high temperatures stress.

Table 7: Foliar application of chitosan on heat stress in various crops

Plant	Method of application	Mode of action/Effects	References
Tomato ( <i>Solanum lycopersicum</i> L.)	0.003, 0.006, 0.009, 0.012% chitosan, foliar application	Improving the growth and quality attributes of tomato under plastic tunnel condition	Hussain et al. (2019)
Cayenne pepper plant ( <i>Capsicum annum</i> L.)	0.00125, 0.00250, 0.00375% Chitosan, plant spraying	Chitosan application permits vegetative growth in unheated greenhouse conditions	Al- Hassani and Majid (2019)
Egg plant ( <i>Solanum melongena</i> L.)	0.0125, 0.0150, 0.02% Chitosan, foliar application	Linear electron flow and non-photochemical quenching can be increased by chitosan application	Liaqat et al. (2019)



## 7. Conclusion

Currently in different sectors of agriculture Chitosan is using as antifungal, bio pesticide and biofertilizer etc. which will be quite useful for adopting sustainable agriculture. Chitosan induced defence response in plants by signal transduction pathway and transduces secondary molecules of  $H_2O_2$  and NO to scavenge ROS against biotic and abiotic stresses. The role of chitosan in the response to abiotic stresses has not been extensively studied therefore, additional research will be needed.

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