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CHITOSAN: SOURCES, EXTRACTION, ANTIMICROBIAL MECHANISMS AND APPLICATIONS IN ORNAMENTAL CROP PROTECTION AND POST-HARVEST MANAGEMENT- A REVIEW

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ABSTRACT
Chitosan, a biodegradable biopolymer derived from crustacean shells, has garnered significant interest as a sustainable solution for plant disease management and postharvest preservation. In ornamental crops, diseases and microbial contamination greatly diminish plant quality and vase longevity. This review gives an overview of chitosan's sources, extraction methods, antimicrobial mechanisms, and uses in ornamental horticulture. Chitosan has a wide range of antimicrobial effects on fungi, bacteria, and viruses. Chitosan also acts as an elicitor, triggering plant defence responses, making plants more resistant to pathogens. Using it as a foliar spray, seed treatment, soil amendment, or vase solution has been shown to improve plant growth, reduce disease, and extend the vase life of cut flowers. But the antimicrobial effectiveness of chitosan depends on things like molecular weight, the level of deacetylation, etc. Chitosan is an eco-friendly way to improve the health of plants and the quality of ornamental crops after they are harvested.

KEYWORDS: Chitosan; Ornamental crops; Antimicrobial activity; Plant defense elicitor; Post-harvest preservation

1. INTRODUCTION

In India, floriculture is a long-standing farming practice with enormous potential for creating profitable self-employment among marginal and small-scale farmers(1). Post-harvest losses are a significant and global problem in the floriculture industry. Flowers are susceptible to substantial post-harvest losses due to their perishability(2). Because of their perishable nature, there is a significant post-harvest loss of between thirty and fifty percent(3). The productivity as well as the quality of many ornamental crops are greatly reduced by plant diseases, which have significant economic implications. Numerous microbial species, such as bacteria, fungi, and viruses, infect ornamental plants in general and negatively impact their growth and appearance, which in turn affects their market value. Both the apparent symptoms of disease and the effects of pathogens on growth have a significant impact on the quality of the flowers (4). Pests such as mites, thrips, aphids, whiteflies, mealybugs, lepidopteran larvae, fungus gnats, and coleopterans are among the primary causes of quality loss during the cultivation of flowers and ornamental plants.(5). Fertilizers and pesticides are among the expensive agrochemicals used in floriculture, and overuse of them leads to the development of pathogens that are resistant to them (6). It has, therefore, become imperative to develop a renewable, inexpensive, and eco-friendly alternative to manage biotic stress and post-harvest loss of ornamentals without producing any disturbing impact on their quality. Biopolymers like cellulose, starch, gelatin, and chitosan have received a lot of attention lately because they have beneficial characteristics, including biodegradability, biocompatibility, and safety for humans (7). Among the biostimulants known for their efficacy, one is chitosan, an amino polysaccharide derived from insect cuticles, fungal cell walls, or seafood shells (8). This polymer functions as an antibacterial agent, antitranspirant, elicitor, and stimulator of plant and beneficial microorganism growth (9). This review intends to evaluate the scientific research gathered on the efficacy of chitosan and its derivatives in addressing biotic stress, minimising postharvest losses, and enhancing growth and flowering in ornamental plants.

Chitosan

Chitin and its deacetylated derivative, chitosan, constitute a group of linear polysaccharides formed from differing quantities of (β1→4) linked residues of N-acetyl-2-amino-2-deoxy-D-glucose (glucosamine, GlcN) and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc) residues(10). Commercial chitosan is made by processing shellfish, shrimp waste, crab, and lobster with strong alkalis at high temperatures for extended periods of time. Due to potential benefits over the present marine source in terms of uniform polymer length, high degree of

deacetylation, and solubility, the manufacture of chitin and chitosan from fungal sources has drawn more interest in recent years(11). However, when chitin is transformed into chitosan, a chitin derivative, its usefulness is enhanced. Since chitosan contains amine groups, it can undergo structural changes that produce a functional derivative of chitin. Chitosan has several uses outside of agriculture, including in the culinary, cosmetic, textile, and biomedical sectors(12).

2. Sources of chitosan

2.1 Chitosan derived from Crustaceans

Nowadays, marine organism waste, mostly the exoskeletons of crustaceans, is the main source of large-scale chitin and chitosan(13). For decades, numerous sources of chitin from crustaceans have been recognised, and new sources are continuously being found. Chitin and chitosan were successfully extracted from shrimp shells by chemical treatment(14). A similar study states that chitin is usually extracted from the exoskeleton of crustaceans, particularly crabs and shrimp, where α-chitin is produced. β-chitin is a more deacetylated type of chitin. Squid is a vital supplier of this type of chitin. It is economical to gather crustacean shells as food waste, especially if carotenoids are recovered(15). Each crustacean organism has a different quantity of chitin; estimates for chitin extracted from crustacean crabs range from 60 to 70 percent(13).

2.2 Chitosan derived from Fungal sources

After crustaceans, fungi are the second most prevalent source of chitin. (16). The composition of chitin, which makes up about 1–15% of the weight of fungal cell walls, is comparable to that of crustaceans. Extreme conditions are required to extract chitosan from fungal chitin, which is similar to chitin from crustaceans. Among the most examined species for direct chitosan processing are *Absidia spp*. (Zygomycetes), *A. niger* (ascomycetes), *Rhizophusoryzae* (zygomycetes), and *Lentinus edodes* (basidiomycetes). By bubbling through potassium hydroxide (KOH), the fungi were rendered pure. By bubbling through potassium hydroxide (KOH), the fungi were rendered pure. It has been stated that this procedure helps remove pigments and proteinaceous materials(8). *Auricula bisporus* (button mushroom), *Lentinus edodes* (shiitake), *Pleurotus spp* (oyster mushrooms), *Flamulina velutipes* (winter mushroom), and *Volvariella volvacea* (straw mushroom) are the most commonly cultivated mushrooms for chitin synthesis(Aida et al., 2009). However, the demineralisation procedure is not necessary for the manufacture of chitosan from fungal sources.

3. Extraction of chitin and chitosan

3.1 Chemical method: This extraction method involves three important phases, such as demineralization, deproteinization and deacetylation(17, 18).

3.1.1 Demineralization: Demineralization is the initial phase involving the removal of inorganic minerals such as calcium carbonate and calcium phosphate from the crustacean shells, forming a porous structure which can then undergo the processes of deproteinization and deacetylation to produce chitosan (19).

3.1.2 Deproteinization: Proteins are removed by an alkaline process using a diluted sodium hydroxide (NaOH) solution. After filtering the mixture and repeatedly washing it with deionised water to remove any residual NaOH, it is dried overnight in an oven. Purified chitin is the name given to the final product(20).

3.1.3 Deacetylation: This procedure involves removing the acetyl group from chitin to transform it into chitosan. The production of chitosan is commonly performed by treatment with a concentrated sodium or potassium hydroxide solution at elevated temperatures. Following the reaction, the material is dried in an oven for the entire night after being repeatedly cleaned with distilled water until it is neutral(21).

3.2 Biological method: Biological methods of chitin extraction can be categorized as enzymatic methods and fermentation methods. As the names suggest, the former breaks down crustacean shells using enzymes, while the latter breaks down the shells using bacteria until only chitin is left(22). Biological processes often yield chitin with a high molecular weight and a low degree of deacetylation, but they have difficulty in fully demineralising and deproteinating chitin(22).

3.2.1 Enzymatic methods

Proteinases are used in enzymatic procedures to separate chitin and eliminate shell proteins. To extract chitin, proteinases are typically obtained from bacteria or marine life's guts(22, 23). Enzymatic techniques remove proteins with little deacetylation and chitin chain damage by taking advantage of the specificity of enzymes and mild reaction conditions (typically 25–59 °C)(22, 24).

3.2.2 Fermentation method

When scaling up reactions, fermentation techniques reduce costs by replacing enzymes with bacterial cultures. In contrast to enzymatic extraction, fermentation techniques can use acid-producing bacteria to incorporate demineralization; lactic acid-producing cultures are frequently employed for this purpose(13, 22). Certain lactic acid-producing bacterial species have poor deproteination capabilities; to increase the purity of chitin generated, fermentation with cultures that produce proteases can be used(25, 26). Protease-producing bacteria frequently

need an extra demineralization step and seldom produce sufficient demineralization on their own(26). The main problem with fermentation technologies is the long fermentation time(27).

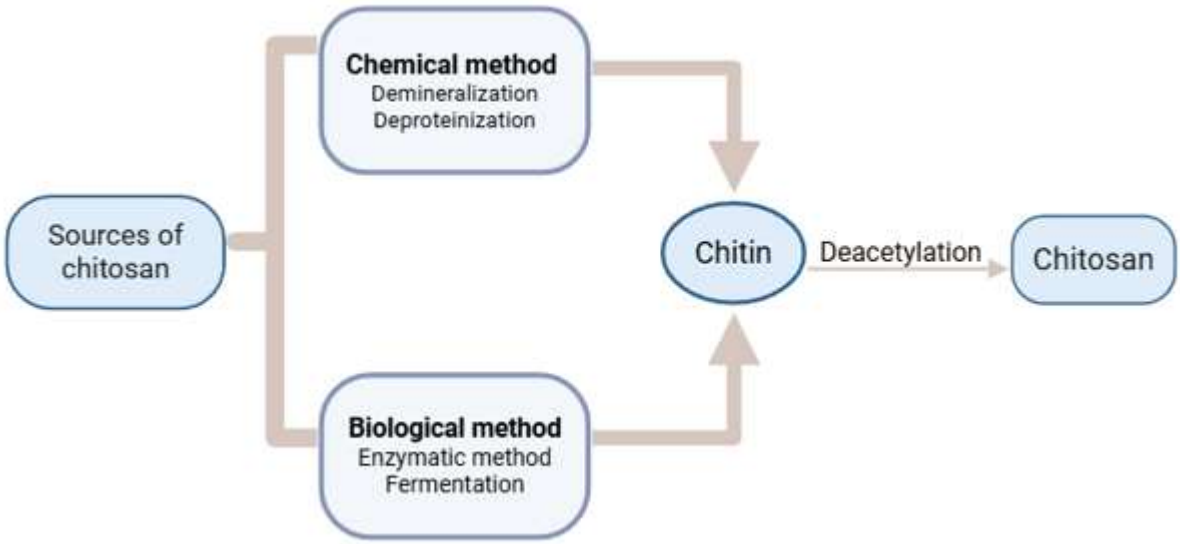

Fig 1: Extraction of chitin and chitosan

4. Antimicrobial activity of chitosan

Chitosan have been examined as an antibacterial material against a wide range of target species, such as algae, bacteria, yeasts and fungi in investigations involving in vivo and in vitro interactions with chitosan in different forms (solutions, films and composites)(28). Numerous studies have documented the antibacterial and plant-defence eliciting properties of chitosan and its derivatives(29). Chitosan treatment has been shown to increase plant resistance to pests and pathogens through the plant-defence-eliciting properties of chitosan and its derivatives under biotic stress (30). In addition to the reactive hydroxyl groups at the C-3 and C-6 positions, chitosan's antibacterial qualities are closely linked to its structure, physicochemical traits, and environmental circumstances(31, 32). Chitosan's antibacterial effects on microorganisms can be categorized as either extracellular, intracellular, or both, depending on where they target(33, 34). Chitosan's antibacterial action, however, is largely reliant on the kind of microorganism it targets(35).

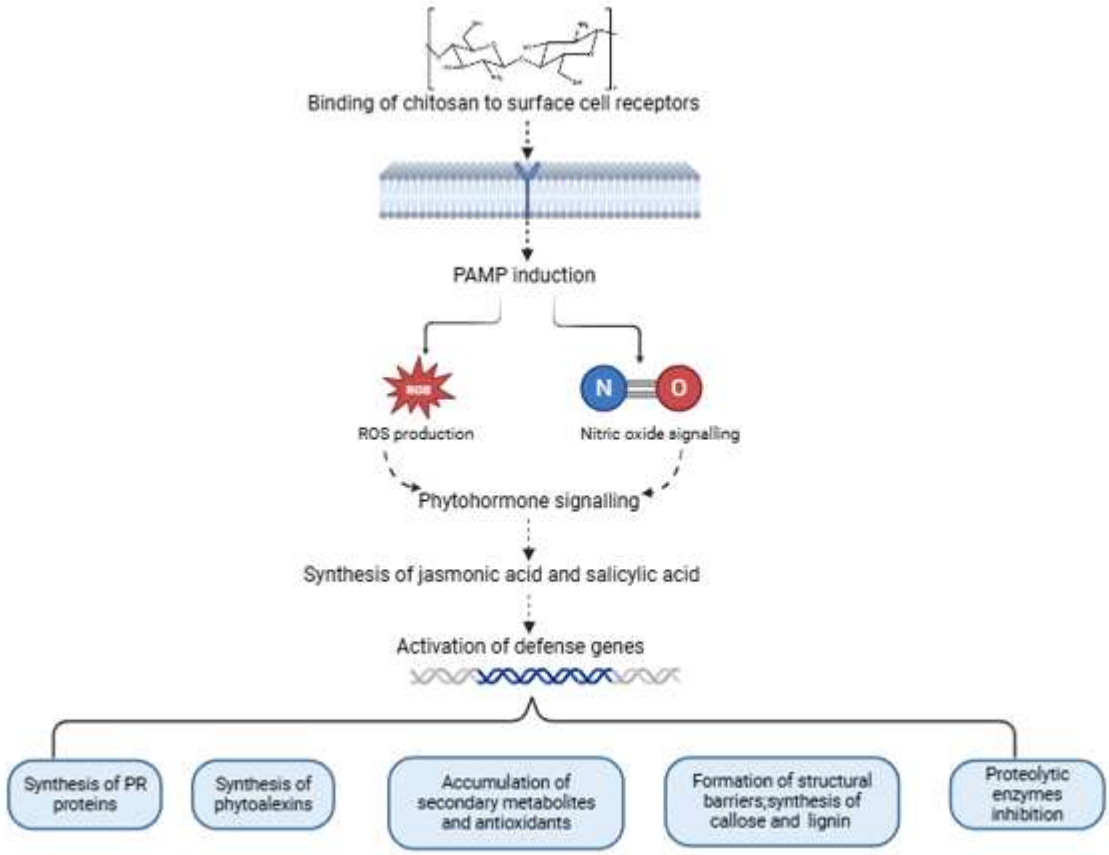

Fig 2: The effect of chitosan on plant defense mechanisms

4.1 Antifungal activity of chitosan

Chitosan's distinct physicochemical characteristics, biodegradability, and biocompatibility give it a potent antifungal activity(36, 37).Chitosan has been used to directly prevent the growth of certain fungal diseases on crop plants due to its biological activity(38) in addition to indirectly promoting defensive mechanisms in plants(39). Several characteristics, including the source of chitin (fungi or crustaceans), an average degree of deacetylation, molecular weight and the range of these values in a specific batch of chitosan, impact the antifungal activity(40).Different fungal species respond differently to chitosan treatments, and the sensitivity changes according to the stage of the fungal life cycle(41).

The antifungal activity of chitosan has been demonstrated through several mechanisms. These include cytoplasmic leakage, chelation of essential nutrients, local disintegration of fungal cell membrane, and nucleic acid binding that modifies the genetic information flow(42, 43). Simultaneously, mycelium growth, the sporulation stage, spore viability and germination efficiency, and the fungus capacity to create virulence factors were all shown to be suppressed by chitosan(44).

Chitosan-containing soil amendments have been demonstrated to reduce fungal infections in a variety of crops, particularly Fusarium wilt and gray mold(45). Part of the effect observed with chitosan on the reduction of soilborne pathogens stems from its enhancement of plant defence responses. The other part is linked to the fact that it is composed of polysaccharides that stimulate the activity of beneficial micro-organisms in the soil, such as *Bacillus*, *Pseudomonas*, actinomycetes, mycorrhiza, and rhizobacteria. Beneficial organisms, on the other hand, can compete with them through mechanisms such as parasitism, antibiosis, and induced resistance(46). As with bacteria, the activity of chitosan against fungi is thought to be fungistatic rather than fungicidal, and it may be possible to transfer regulatory changes in both the fungus and the host. Chitosan's antifungal activity comes from its ability to bind to and stop the production of toxins and the production of many secondary fungistatic metabolites, such as phytoalexins, abscisic acid, methyl jasmonate, and phenolic compounds, as well as various enzymes like hypha-degrading chitinase and β-glucanase(47, 48).

4.2 Antibacterial activity of chitosan

Chitosan and its derivatives suppress the growth of several bacterial plant pathogens(49, 50). According to the evidence that is currently available, fungi seem to be more susceptible to chitosan's antimicrobial effects than bacteria(51). The most widely accepted antibacterial activity of chitosan is attributed to electrostatic interactions that alter membrane permeability. After that, it attaches itself to DNA and prevents DNA replication, which kills

bacterial cells(52). Another possible mechanism is chitosan's ability to chelate metal ions, which increases the synthesis of toxins and inhibits bacterial survival(53). In acidic environments, chitosan exhibits higher chelating activity toward a variety of metal ions, including Fe^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+}. The stability of bacteria depends on metal ions that are linked to their cell wall polymers(54).
The bacterial target (gram-negative (G−) versus gram-positive (G+) bacteria), development stage, zeta potential, concentration, pH, molecular weight, and degree of deacetylation are some of the variables that cause chitosan-nanoparticles to have antibacterial action(55). Although some research indicates that gram-positive bacteria are more sensitive to chitosan(56), but it has been suggested that gram-negative bacteria may be more vulnerable to it than gram-positive bacteria(57, 58). Furthermore, gram-positive bacteria have a thicker cell wall than gram-negative bacteria, which may hinder chitosan's ability to attach directly to the cell membrane. Nevertheless, certain chitosan oligomers (less than 5 kDa) can pass through the cell wall and impact the synthesis of proteins or DNA/RNA(59, 60). Remarkably, studies have shown that chitosan (≤50 kDa) can block DNA transcription by penetrating the cell wall(59). Therefore, even though chitosan's molecular size is crucial for targeting, its extracellular, intracellular, or both extracellular and intracellular antibacterial action is also determined by its structure rather than its molecular weight.

4.3 Antiviral activity of chitosan

Plant viral infections are controlled by chitosan(61). The majority of the plants treated with chitosan did not experience systemic viral infection because chitosan increased plant resistance to viral diseases and prevented viruses and viroids from spreading(62). The average degree of polymerization, the degree of N-deacetylation, the positive charge value, and the nature of the molecule's chemical changes all impact chitosan's antiviral activity(63). By simulating the plant's interaction with a phytopathogen, chitosan triggers a variety of defense mechanisms that prevent viruses and viroids from spreading. Phage particle inactivation and suppression of cellular bacteriophage reproduction are key components of chitosan's ability to reduce phage infections(62).

5. Insecticidal property of chitosan

Certain chitosan derivatives have shown insecticidal activity against a range of agricultural pests, and chitosan itself can improve the availability and stability of certain insecticides or botanicals (64). As a safe and environmentally acceptable substitute for synthetic pesticides, chitosan is currently approved in the EU as an active and non-toxic basic chemical for plant protection (Regulation EC No 1107/2009)(65, 66). Specifically, the polymer has been used in several formulations, including coated chitosan, chitosan derivatives, chitosan–metal complexes, chitosan nanoparticles loaded with essential oils and insecticides, and a simple chitosan formulation sprayed on leaves, wood, and paper(67). By reducing their volatility, chitosan nanoparticles containing essential oils can increase their stability and bioactivity. In fact, adding well-known essential oils with insecticidal and insect-repellent properties to chitosan enhances its bioactivity. The effectiveness, durability, and availability of essential oils are enhanced by the antifeedant and antitoxic properties of chitosan coated with various essential oils (67).

6. Factors affecting antimicrobial activity of chitosan

6.1 pH

At pH less than 6, chitosan is polycationic and easily interacts with negatively charged materials such as proteins, fatty acids, bile acids, anionic polysaccharides, and phospholipids because of the polymer's high density of amino groups(68). When the glucosamine monomer's NH3+ group has a positive charge at pH less than 6.3, it can interact with negatively charged microbial cell membranes to cause internal components to leak out. Because chitosan's amino groups ionize at pH values lower than 6, its antimicrobial action is higher at low pH values(69). Due to its limited water solubility and amino group deprotonation, chitosan lacked antibacterial action at pH 7.0. Chitosan's amino groups have pKa values between 6.3 and 6.5, implying that as the pH rises above 6.5, it becomes insoluble in water, organic solvents, and alkaline solutions. In addition, as the pH of the solution drops, the solubility rises, increasing the positive charge on the chitosan's -NH3 groups and boosting its antibacterial action(35, 59).

6.2 Molecular weight

The ability of chitosan to exhibit intracellular antibacterial action through cell surface penetration is determined by its molecular weight(70). The three types of chitosan are high molecular weight, low molecular weight, and oligochitosan, which can be identified by their molecular weight(71). Because high molecular weight chitosan cannot cross the microbial barrier, it stacks on the cell surface and prevents nutrients from entering the microbial cell membrane, which causes cell lysis(72)However, in solution, fragmented chitosan molecules with a smaller molecular weight (<5000 kDa) may attach to DNA and prevent mRNA synthesis by entering the microorganisms' nuclei(71, 73).

6.3 Degree of deacetylation

The amount of free amino groups in polysaccharides is determined by the degree of deacetylation. The degree of deacetylation is commonly known as one of the most crucial chemical properties that may affect chitosan's effectiveness in a variety of applications is the degree of deacetylation(74). The preparation technique, namely the processing duration and temperature employed during chemical treatment, has a significant impact on the DDA of chitosan(75). A high DDA is typically the result of longer processing periods and higher temperatures(76, 77). Additionally, it has been demonstrated that in the same acidic environment, chitosan with a high DDA shows a greater positive charge than chitosan with a low DDA(76). Therefore, chitosan with a high DDA interacts with the microbial cell surface more strongly through electrostatic forces, which frequently leads to improved antimicrobial activity.

6.4 Derivatives

Despite molecular weight, degree of deacetylation, and pH all having an impact on chitosan's antibacterial action, the physicochemical properties of the C2-NH2, C3-OH (secondary hydroxyl), and C6-OH (primary hydroxyl) functional groups of chitosan also have a major impact. As a result, many chitosan derivatives modified with amine (N-modified) and hydroxyl (O-modified) groups via acylation, carboxylation, alkylation, and quaternization have been produced and studied because of the low solubility and lack of a positive charge at neutral pH(57, 76). Numerous N-modified chitosan derivatives have shown enhanced antibacterial properties, including quaternary ammonium chitosan, imino-chitosan, acetylphenyl-thiosemicarbazone, N-benzyl and thymine-based chitosan, and alkyl sulfonated derivatives(78).

7. Antimicrobial mechanism of chitosan

The mode of action of chitosan against microorganisms can be divided into extracellular, intracellular, or both, depending on where the antimicrobial effects are targeted(35, 78). Chitosan triggers defense mechanisms and reactions to pathogen attacks. The presence of chitosan promotes the production of hydrogen peroxide, glucanases, chitinases, pathogenesis-related proteins (PR), phytoalexines, and protein inhibitors(79). The one known mechanism is the (1) electrostatic interaction in which the negatively charged pathogen surfaces may interact with the positively charged chitosan molecules, which could destroy the cell structure, alter the cell surface extensively, and increase membrane permeability(80). This would allow intracellular substances to leak out and eventually impair the pathogen's vital functions(81). It was discovered that chitosan reacted with both the cell wall and the cell membrane, but not simultaneously. This suggests that chitosan inactivates pathogens through a two-step sequential mechanism, whereby the cell wall first detaches from the cell membrane, and then the cell membrane is destroyed (82). (2)The development of an impermeable layer of chitosan polymer on the pathogen cell surface inhibits both the excretion of metabolites into the intercellular space and the absorption of nutrients(83). (3) Chitosan is believed to have the ability to attach to DNA and prevent messenger RNA (mRNA) synthesis by penetrating the nucleus of the microbes and obstructing the production of proteins and mRNA. (4) Chitosan can chelate metal ions and some minerals required for bacterial or fungal growth, preventing the latter from reproducing and producing toxins (73, 83).

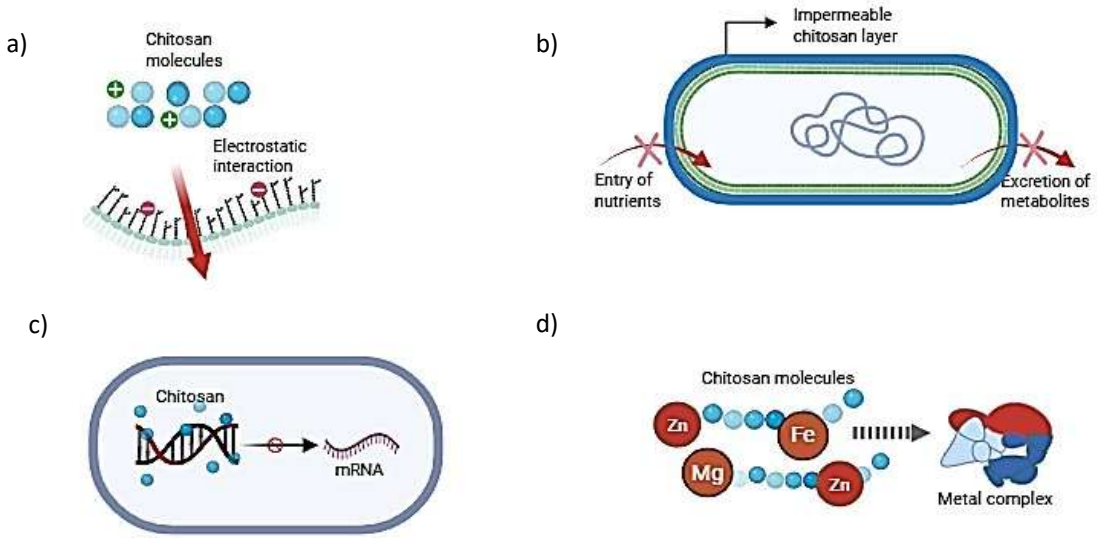

Fig 3: Antimicrobial mechanism of chitosan

7. Methods of application of chitosan

Foliar spray: The most commonly employed method in ornamental horticulture is the weekly application of chitosan hydrochloride, which is a basic compound (0.05 g m^{-2}). This treatment significantly reduced the infected leaf area of cutting roses and French hydrangeas caused by *Podosphaera pannosa* and *Erysiphe polygoni* compared to water-treated controls(84).
Soil drench/incorporation – Chitosan solution (5%) incorporated into the substrate has shown to decrease soil pH and conductivity, reducing *Alternaria* (20%) and *Fusarium* (50%) inoculum, and at the same time promoting *Purpureocillium*, which offers protection to potted ornamental plants(85).
Seed/seedling priming – Treatment of seeds with chitosan and chitosan nanoparticles (0.05-0.1% w/v) has been shown to increase germination rates and prime antioxidant enzyme activities, which offer protection against fungal infection when the seedlings are transplanted(86).

Table 1: Effect of chitosan on disease management in ornamentals

Sl no	Flower crop	Disease	Concentration	Effect	Reference
1.	Gray mold	*Begonia × hiemalis* 'Schwabenland Red'	0.10 g L^{-1} chitosan + N:P:K = 2.8:1.0:1.4 (drip)	Disease severity decreased to 0.17%, incidence 0 %; increase in plant height, crown development, earlier flowering, larger & more flowers; increased activities of SOD, POD, CAT, PAL.	(87)
2.	Bacterial leaf spot	*Euphorbia pulcherrima*	0.10 mg mL^{-1} chitosan (pH 5.5-7.0);	> 3.86 $\log_{10}$ CFU mL^{-1} reduction after 6 hour; activity rises with NaCl.	(88)
3.	Powdery mildew	*Calendula officinalis*	50 mg L^{-1} chitosan, three foliar applications	Severity decreased to 18 % (greenhouse) and 26-27 % (field) vs. 45-62 % control.	(89)
4.	Powdery mildew	Cutting roses ('Beluga', 'Susan') & French hydrangea	0.05 g m^{-2} chitosan HCl, weekly foliar spray	Decrease infected leaf area; effective at low disease levels; no phytotoxicity.	(84)
5.	Gray mold	Petunia	0.4 % reagent-grade chitosan, foliar spray	Lesion size reduced up to 60 %; no phytotoxic effects.	(90)
6.	Powdery mildew	*Rosa roxburghii* Tratt.	1.0-1.5 % chitosan, foliar spray (≈1.5 L $plant^{-1}$, twice)	Control efficacy 69-73 %; increase in SOD, POD, flavonoids, proline, soluble sugars content; decrease in MDA content.	(91)
7.	Powdery mildew	*Rosa roxburghii* Tratt.	Pyraclostrobin 100 mg L^{-1} + chitosan 500 mg L^{-1}, foliar spray (twice)	Control efficacy 89-95 %; increase in chlorophyll content, photosynthetic rate, fruit weight and yield.	(92)

8. Role of chitosan in extending the vase life of flowers
Despite the vast economic magnitude, postharvest losses remain one of the most critical challenges facing the cut flower sector. The magnitude of loss in the cut flower sector is estimated at around 20-40% due to inefficient postharvest packaging and storage. Postharvest handling is vital in ensuring the quality and shelf life of cut flowers, given their transient nature(93). The main causes of cut flowers' shortened vase life are nutritional deficiencies, bacterial and fungal contamination(94), water stress-induced wilting, and vascular blockage(95).One of the most important advantages of chitosan over traditional methods is its multifunctionality. Traditional preservatives usually work on only one main mechanism: sucrose replenishes energy substrates; Silver thiosulphate and 1-Methylcyclopropene inhibit ethylene; 8-Hydroxyquinoline sulphate and 8-Hydroxyquinoline citrate inhibit bacterial growth. Chitosan, on the other hand, works on multiple deterioration mechanisms

simultaneously in one application. It acts as an antimicrobial, antioxidant, senescence retarder, membrane stabilizer, and plant defense elicitor(48, 96).

8.1 Factors affecting the vase life of flowers

The vase life of cut flowers is majorly affected by pre-harvest, during harvest and post-harvest factors.

8.1.1 Pre-harvest factors

The primary pre-harvest factors influencing vase life in cut flowers are genetic makeup and growth circumstances. Maximizing the post-harvest endurance of flowers requires an understanding of the ideal environmental factors and genotype interaction(97).The environmental factors that affect vase life are light, temperature, and humidity(98).

8.1.2 During harvest factors

Harvest time affects the post-harvest durability of cut flowers. Determining the harvest time and method for harvesting is crucial. The initial impact of the flower's harvest stage is on the flower, followed by the flower opening(99). Flowers wilt and lose a lot of water when harvested at midday. The morning harvest is more advantageous than the midday harvest since the temperature and relative humidity are lower in the early morning, and the plant has a higher water content(100).

8.1.3 Post-harvest factors

Cut flower post-harvest physiology examines the functional processes that occur in plant material following harvest or removal from its native growing environment until it is used or decomposes.Some of the post-harvest factors affecting vase life of cut flowers are water uptake, water quality, ethylene sensitivity, ventilation, packaging, etc(98).

8.2 Causes of vase life deterioration in cut flowers

8.2.1 Vascular Occlusion and Water Relations Impairments

Wound xylem occlusion is a major factor that influences the vase life of cut flowers. Oxidative stress and phenolic compound polymerization result in the accumulation of phenolic compounds and secondary metabolites in the stem ends of cut flowers to close open tissues of freshly cut stems and protect against microbial entry. Yet, this process results in vessel blockage, decreased water absorption, and shortened vase life. Physiological vessel plugging is associated with the activities of diverse oxidative enzymes, namely phenylalanine ammonia-lyase, polyphenol oxidases, laccases, and class III peroxidases, which enhance the production of diverse compounds like lignin, suberin, tyloses, gel, and latex(101).

8.2.2 Microbial growth and stem end blockage

Pathogen pressure can significantly reduce the vase life by affecting the water balance negatively due to the presence of bacteria in the vase water(102). Previous studies have shown that when the number of bacteria in the vase water exceeded 10^7 CFU/mL, there was a decrease in the hydraulic conductance, flower life, fresh weight, and quality of flowers(103).

8.2.3 Carbohydrate depletion

Cut flowers are excised from organs like bulbs, tubers, roots, and stems that serve as a source of carbohydrates during harvest. After harvest, cut flowers need continued growth and metabolism. Plant produce is typically transported and stored in the dark, where photosynthesis is restricted, and metabolism depletes carbohydrates(102).

8.3 Mechanisms of chitosan in extending the vase life of cut flowers

8.3.1 Antimicrobial action and prevention of xylem blockage

The positively charged amino groups of chitosan interact with the negatively charged cell membranes of microbes, resulting in the leakage of cell contents, damage to cell membranes, and the inhibition of DNA/RNA and protein synthesis, ultimately resulting in microbial cell death(104). The antimicrobial properties of chitosan decrease the growth of bacteria, yeast, and molds in vase solutions and on stem ends, thus preventing xylem blockage and increasing water transport(105, 106).

8.3.2 Antioxidant defense and protection of the cell membrane

Chitosan and chitosan nanoparticles can enhance the activities of antioxidant enzymes and reduce oxidative damage, thereby delaying petal senescence. In cut roses, the treatment with nanochitosan showed enhanced activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase, with significant reduction in the levels of hydrogen peroxide(H_2O_2) and malondialdehyde (MDA), and membrane integrity indices(107). Comparable increases in the activities of superoxide dismutase (SOD) and catalase (CAT), and a reduction in H_2O_2, were also found when chitosan nanoparticles were added to the tulip vase solution(105).

8.3.3 Hydration and water balance improvement

Chitosan prevents the vascular system from becoming clogged, thereby promoting higher water uptake and a positive water balance. Cut gerbera in a nanochitosan solution had higher relative water uptake and water balance than cut flowers in citric acid or water(108). Chitosan nanoparticles in roses and tulips increased solution uptake, relative fresh weight, and petal water content, which was reflected in the longer vase life(109).

8.3.4 Induction of secondary metabolites and reinforcement of structural integrity
Chitosan has an eliciting effect, which enhances the production of phenolic compounds, flavonoids, anthocyanins, and other secondary metabolites(110). Chitosan has been reported to trigger the expression of phenylalanine ammonia-lyase and other defense enzymes, which can improve the lignification process and may delay the deterioration of the vision in the petals and bracts(64, 109).
8.3.5 Modified gas/moisture exchange and a semi-permeable coating
Chitosan coating or film on ornamentals and flowers can thus induce a modified microatmosphere around tissues, hence reducing physiological stress and increasing display life(111, 112).When chitosan is applied as a coating and not as a vase solution, it forms a semipermeable film that limits the diffusion of O_2, CO_2, and water vapor(113).

8.4 Methods of application of chitosan for extending vase life
8.4.1 Chitosan in the vase/holding solution
Cut stems will be placed continuously in a chitosan or nano-chitosan vase solution. Nano-chitosan solution inhibited stem bending, enhanced relative water uptake and water balance, and significantly suppressed bacteria, molds, and yeasts in gerbera(96). Chitosan nanoparticles in preservative solution extended the maximum vase life of 15 days, reduced microbial population, enhanced phenolics, flavonoids, anthocyanins, and antioxidant enzymes. They reduced hydrogen peroxide and malondialdehyde in *Rosa hybrida* cv. Black magic (114).
8.4.2 Pulsing
Pulsing with nanomaterials is emphasized in a current meta-analysis as a broadly effective approach, where nanomaterials (including nano-chitosan) are advised as a first pulse treatment, followed by species-specific vase solution(115). In chrysanthemum, chitosan was used as a pulse treatment (25-100 mg/L for a short period) either alone or in combination with sucrose and/or Calcium nitrate.
8.4.3 Surface coating of inflorescences
Dipping *Heliconia bihai* stems in a 1.0-1.5% chitosan solution prolonged vase life by 10.3 and 7 days, respectively, compared to controls. The coated inflorescences showed reduced fresh weight loss, improved bract colour, increased anthocyanins, flavonoids, and sugars, as well as improved membrane integrity from the stem base to the bracts(112). Spray treatment of carnation petals with 0.1% chitosan solution increased shelf life by about 3 days compared with uncoated controls, as it reduced water loss, weight loss, and colour change during storage(116).

Table 2: Effect of chitosan on vase life extension in ornamentals

Plant species/cultivar	Chitosan form	Method of application	Effect on vase life	Reference
Tulip	Nano-chitosan	Vase solution	13.01 and 14.00 days in two seasons; clearly longer than the control	(108)
Rose(*Rosa hybrida* 'black magic')	Chitosan nanoparticles	Preservative vase solution	Maximum vase life 15 days at 10 mg/l; longer than control	(114)
Gerbera (*Gerbera jamesonii* 'Terra kalina')	Nanochitosan encapsulated melatonin	vase solution	Longest vase life 12 days at 0.1 Mm;better than control and free melatonin	(109)
Carnation (*Dianthus caryophyllus* 'Tabor')	Chitosan	Vase solution	Vase life extended by 10.3 days compared with control	(117)
Heliconia bihai 'halloween'	Chitosan	Stem coating/dip	Vase life extended by 10.3 days in 1% and 7 days in 1.5% compared with the control	(112)
Rose (*Rosa hybrida* 'morden fireglow')	Nano-encapsulated arginine+ phenylalanine	Vase solution	Maximum longevity 16.3 days at 5 mg/l than control	(118)
Alpinia purpurata 'pink'	Chitosan	Quick dip before holding	Vase life extended	(119)

Chrysanthemum 'Pingpong' group	Chitosan	Vase solution	Chitosan significantly extended the shortened vase life	(120)
Gladiolus 'white prosperity'	chitosan	Vase solution	Vase life extended than the control	(121)
Anthurium	Chitosan with sucrose	a.Preservative solution b.spray method	Maximum vase life 53 days in 0.2%chitosan+2% sucrose Maximum vase life 35 days in 0.2%chitosan+2% sucrose	(122)

9. Plant protection products based on chitosan

KitoGreen Master/Direct, a chitosan derived from fungi and formulated as a 5 % solution, has been shown to lower soil pH and conductivity, and to control *Alternaria* inoculum levels by approximately 20 %, *Fusarium* inoculum levels by approximately 50 %, and the fungus *Purpureocillium* levels 50-fold(123). Chitomax, part of the Adler Agro group, also offers chitosan hydrochloride solution, which is effective against *Botrytis* and Phytophthora infestans and has been shown to directly damage bacterial membranes(66). Sela Chito offers chitosan oligosaccharide, which is effective at 2.5 % w/w and synergistically controls Botrytis with synthetic fungicides, and induces lignin, phenolic, and cellulose compounds(124). Spectrum CHI (Indigrow) provides a 10% w/v chitosan oligosaccharide solution; its use as a foliar spray resulted in ISR marker gene expression (PR-proteins, SA and JA pathways)(125). Chito Pure (Nature) offers a chitosan hydrochloride solution; it controls post-harvest diseases through a three-fold mode of action involving elicitation, antifungal activity, and film formation(126). Serbios' water-soluble chitosan hydrochloride preparation shows direct antifungal activity against Fusarium, *Botrytis*, and *Alternaria* spp., and stronger activity against Gram-positive bacteria(127). Lastly, ChitoLytic COS (ChitoLytic) contains high-purity low DP chitosan oligosaccharides, which displayed the highest antifungal activity and synergised with low-dose synthetic fungicides(124).

10. Chitosan effects on gene expression

Chitosan improves biotic stress management in ornamental plants by upregulating genes related to plant defense responses, including pathogenesis-related proteins such as glucanase, chitinase, antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). The upregulation of these genes improves plant immunity against pathogens, including fungi and bacteria, through signalling pathways mediated by reactive oxygen species (ROS), nitric oxide, and plant hormones, including jasmonic acid and salicylic acid(128, 129). Targeted chloroplast gene modulation: In *Dendrobium* 'Eiskul', chitosan O-80 (10-50 ppm) down-regulated the chloroplast ycf2 gene expression in 12-48 hours, leading to increased chloroplast size and silica-rich vascular bundles due to increased synthesis of defensive compounds.Activation of antioxidant defense genes: Chitosan nanoparticles (10 mg L^{-1}) in vase solutions *for Rosa hybrida* 'Black Magic' extended vase life for approximately 15 days by transcriptionally up-regulating antioxidant defense genes, thereby increasing SOD, POD, CAT, and APX activities(114).Broad-spectrum stress-responsive gene induction: In *Calendula officinalis*, chitosan treatment increased catalase, guaiacol peroxidase, and superoxide dismutase activities by inducing antioxidant gene networks for improved drought resistance and pathogen resistance(130).Secondary metabolite pathway enhancement: In *Microsorum scolopendria*, foliar chitosan treatment up-regulated the MEP/MVA pathway for sterol synthesis by increasing 20-hydroxyecdysone levels for plant defense response and growth promotion(131).

11. Limitations of chitosan

Chitosan's antimicrobial activity is greatly reduced when the pH is above neutral due to the loss of protonated amine groups. This limits the activity of chitosan in soil or vase solutions that usually maintain a pH above 7. Relatively high concentrations of chitosan may be required for effective disease management. For example, to control *Fusarium* spp. on *Schefflera*, the lowest concentration of chitosan that still had a significant effect on the reduction of disease incidence was 25 mg L^{-1}, whereas lower concentrations were found to be almost ineffective(132, 133). The antifungal activity of chitosan is extremely susceptible to the molecular weight and level of deacetylation. Low molecular weight chitosan is more active than high molecular weight chitosan(134). The elicitor effect, which induces the expression of defence genes, can take a long pre-application period (≥24 weeks) to develop the necessary resistance(135). Chitosan solutions, without any additional compounds, are often found to have limited efficacy against diseases, with fungicides achieving better results. To obtain efficacy levels of ≥80% against diseases, synergistic mixtures are necessary, which increases the complexity of the formulation, thereby increasing the cost(134). For the use of chitosan solutions as a vase life extension treatment, the beneficial effect of the chitosan solution as a physical barrier is lost as the solution becomes alkaline over time, which

necessitates the renewal of the solution(135). For the commercial use of chitosan solutions, the lack of complete studies on the safety of the product, including the lack of a standardized toxicity study for chitosan-based nanomaterials, is a cause for concern(136).

12. CONCLUSION AND FUTURE PROSPECTS

Chitosan is a natural biopolymer that has a lot of promise in ornamental horticulture because it can kill bacteria, break down naturally, and make plants defend themselves. If used on ornamental crops, it is effective at controlling pathogens, making plants grow better, and making cut flowers last longer after they are picked. Chitosan is an environmentally friendly alternative to traditional chemical treatments because it stops microbes from growing and boosts plant physiological responses. Chitosan has some benefits, but its effectiveness can change based on things like molecular weight, how much it has been deacetylated, and the conditions in which it is used. Also, there aren't enough standardized application protocols and large-scale evaluations yet. Future research should concentrate on creating enhanced formulations, including chitosan nanoparticles and derivatives, to improve stability and antimicrobial efficacy. More research is needed to improve application methods, test long-term environmental safety, and explore the synergistic combination of biological control strategies with chitosan. Improvements in these domains will make chitosan a more effective and long-lasting way to protect ornamental crops and manage them after they are harvested.

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Conflict of interest

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