Open Access

Contemporary Research Analysis Journal

e-ISSN: 3050-5909
p-ISSN: 3050-5895

Volume 03 Issue 03 March 2026
CrossRef DOI: 10.55677/CRAJ/05-2026-Vol03I03

Page no: 162-168

Mechanism of Fungal Disease Management using Bioextracted Chitin Molecules: A Review

Bashir Alabi Ali[*1,2], Kutama Ahmad Shehu[2], Mohammed Isa Auyo[2], Aisha Idris[2], Maryam Muhammed[2], Adeniyi Kamoru Abdulazeez[3]

[1]Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba, Kogi State.

[2]Department of Plant Biology, Faculty of Life sciences, Federal University Dutse, Jigawa State.

[3]Department of Animal and Environmental Biology, Faculty of Life sciences, Federal University Dutse, Jigawa State.

ABSTRACT: Groundnut (*Arachis hypogaea* L.) is an economically and nutritionally important legume widely cultivated in tropical and subtropical regions, including West Africa. However, its production is significantly constrained by fungal diseases that affect multiple plant parts and result in considerable losses in yield and quality. Existing disease management approaches, such as chemical-based strategies, present challenges related to environmental safety, sustainability, and pathogen resistance, underscoring the need for alternative control measures. Chitin and it's deacetylated derivative, chitosan, have emerged as promising biopolymers for fungal disease management due to their biodegradability, biocompatibility, and broad-spectrum antifungal properties. Chitin, a structural polysaccharide found in fungal cell walls and crustacean shells, can be extracted through chemical or biological processes, while chitosan exhibits enhanced bioactivity owing to its cationic nature. These compounds exert antifungal effects through multiple mechanisms, including disruption of fungal cell wall integrity, inhibition of spore germination and mycelial growth, chelation of essential nutrients, and stimulation of host defence responses. In addition, chitin-based substrates promote the activity of beneficial chitinolytic microorganisms, thereby enhancing biological control. In agricultural applications, chitin and chitosan demonstrate potential as eco-friendly disease management tools through their use as biopesticides, soil amendments, and post-harvest protective agents. Their multifunctional properties and low environmental impact position them as sustainable alternatives or complements to conventional fungicides. Integrating chitin and chitosan-based strategies into groundnut disease management systems may contribute to improved crop health, productivity, and long-term food security.

KEYWORDS: bioextracted chitin, chitosan antifungal, fungal disease management, groundnut diseases, sustainable agriculture

Cite the Article: *Ali, B. A., Shehu, K. A., Auyo, M. I., Idris, A., Muhammed, M., Abdulazeez A. K. (2026). Mechanism of Fungal Disease Management using Bioextracted Chitin Molecules: A Review. Contemporary Research Analysis Journal, 3(3), 162–168.* https://doi.org/10.55677/CRAJ/05-2026-Vol03I03

Publication Date: March 10, 2026

**Corresponding Author: Bashir Alabi Ali*

INTRODUCTION

Human populations rely heavily on consistent and stable agricultural production for economic gain through cash crops and food production to manage food security. One such important food crop is groundnut (*Arachis hypogaea* Linn.), a member of the Fabaceae (Leguminosae) family and the subfamily Papilionoideae, consumed yearly throughout the world. Nigeria, Gambia, Togo, the Republic of Benin, Ghana, the Ivory Coast, Liberia, Chad, Niger, Senegal, Mali, and Guinea-Bissau are the main producers of groundnuts in West Africa. In the region of Nigeria, back in the 1950s and 1960s, there were groundnut pyramids that symbolized the major production of this valuable crop that continues to this day in importance. (Janila *et al*., 2013).

It is an essential legume crop cultivated extensively across tropical and subtropical regions, including Nigeria, due to its adaptability to diverse environmental conditions (Nigam *et al*., 2019). It is a vital source of protein, edible oil, vitamins, and minerals, contributing significantly to human nutrition and food security (Janila *et al*., 2013). Groundnut contains approximately 25–30% protein and 45–50% oil, making it one of the richest sources of vegetable oil and protein among food legumes (Sengupta *et al*., 2022). They serve as a primary food source, especially in the northern states, and hold special value, often referred to as "women's riches" or "women's treasure," in certain regions. These nuts are not just nutritious but also have healing properties, according to Abdulrahama *et al*. (2014).

Numerous fungal infections, including those caused by *Cercospora*, *Alternaria*, *Helminthosprorium*, *Gloeosporium*, *Colletotrichum*, *Septoria*, and *Sphaceloma*, have been reported to cause damage to leaves, stems, roots, and pods, increasing yield loss (Sharma *et al*., 2020).

Plant fungal diseases pose serious threats to food security; this has been greatly researched by authors like Gabisa (2024), who identified the mycoflora associated with groundnut seeds and its impact on seed germination, and Isalar *et. al.*, (2021) worked on fungal contaminants associated with groundnut (*Arachis hypogaea*) seeds. Asama and Chanyya (2020) reported on fungal pathogens of postharvest rot of groundnut (*Arachis hypogaea* L.) in Hong Local Government Area of Adamawa State, Nigeria. Kumar and Thirumalaisamy (2016), evaluated the fungi diseases of groundnut, while Adithya (2016) assessed the Effect of *Aspergillus flavus* on groundnut seed quality and its management, others include Pal *et. al.* (2014); Chigoziri and Temitope, (2020). Various disease recognition techniques are required for the rapid detection of diseases during the early phases of plant growth and fungal infections (Singh and Misra, 2017). The current approach to plant disease recognition is based on the identification and detection of fungal pathogens by comparison with databases from national or regional biological information systems. However, this approach is unreliable. Over the past two decades, plant diseases, such as those that affect leaves, have been investigated using visible array images. Nevertheless, the methodology to date is imperfect and tends to rely upon idealized disease models and scenarios (Barbedo, 2016)

Umma *et al.* (2014) identified eight (8) diseases of fungal rampaging on groundnut causing major losses of groundnut production in Nigeria and identified leaf spot as the topmost disease affecting groundnut in Nigeria. Various control strategies for fungal diseases, including the use of fungicides, have been identified by Sosa *et. al*. (2022); biological control by El-Baky and Amara (2021); cultural practices by Onitilo *et. al.* (2023), technological controls have been identified by Bushnev *et al.* (2023), while effective drugs and novel immunotherapeutic strategies were identified by Brandt and Park (2013). However, a single effective method for the control of fungal diseases has not yet been identified, and as such, researchers are beginning to understudy the molecular pathways of attacking fungi using various means, a reason for which fungal cellular components such as chitin and chitosan are a good bet, as they also serve to protect the fungi themselves from attacks.

Alternative, ecological, and biodegradable materials have been sought after for a variety of scientific uses in recent decades. Biopolymers, including chitosan, cellulose, starch, and gelatins, have drawn a lot of interest because of their beneficial properties, which include non-toxicity to humans, biodegradability, and biocompatibility. Chitosan is the most important derivative of chitin, among other compounds. (Kanmani et *al.,* 2017; Sasikanth et *al.,* 2024)

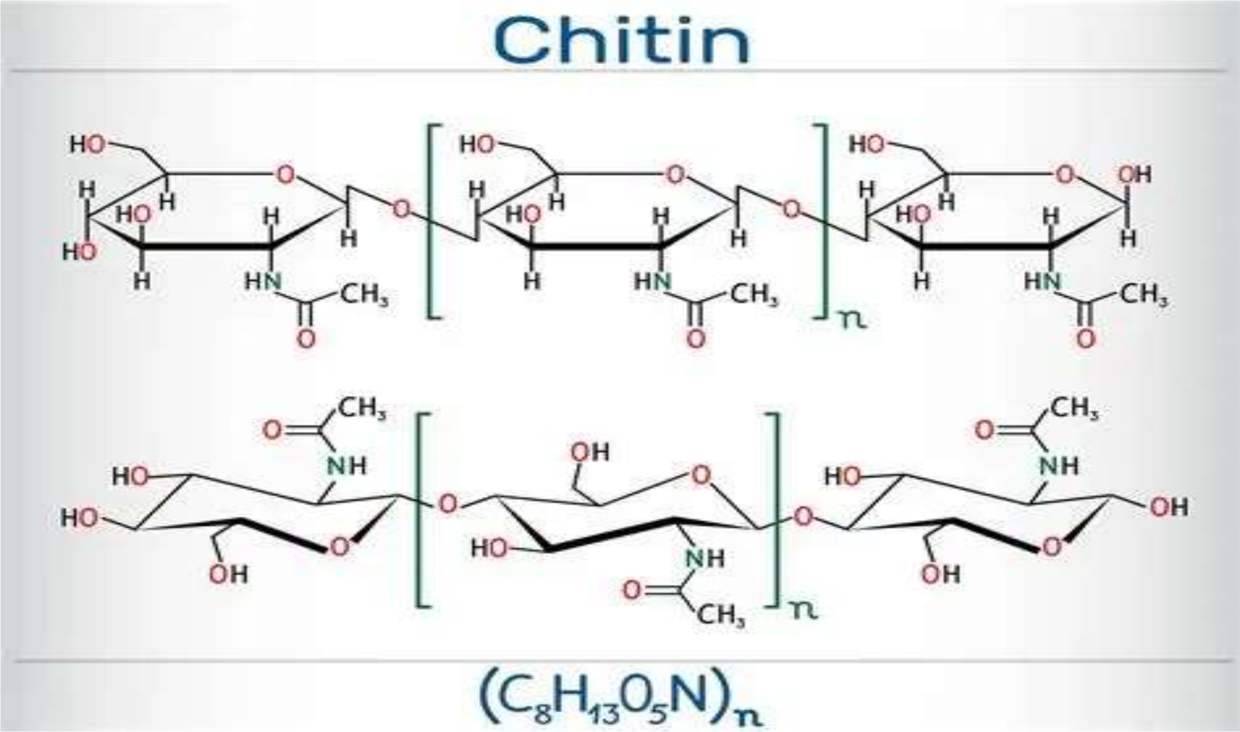
Chitin and Chitosan Molecules.

Chitin is a long-chain polymer of N-acetylglucosamine, a derivative of glucose, and is classed as a structural polysaccharide. It is formed of repeating units linked by β-(1→4) glycosidic linkages, which lend stiffness and stability to its molecular structure (Ahmedd, 2025). This biopolymer is a major part of the cell walls of several fungi and the exoskeletons of arthropods, including insects, crustaceans, and arachnids. The molecular architecture of chitin allows it to form tightly packed crystalline microfibrils, contributing significantly to the mechanical strength and resistance to enzymatic degradation observed in natural chitinous materials. The structure is further stabilized by the acetylamino group's enhancement of intermolecular hydrogen bonds. Owing to these qualities, chitin plays a critical role in defending organisms from external stresses and has earned. Substantial interest in diverse applications, including biomedical engineering and biotechnology.

Extraction methods of chitin and chitosan.

Extraction of Chitin

The extraction of chitin from natural sources involves the removal of proteins, minerals, lipids, and pigments to isolate the pure biopolymer. This process is necessary because raw chitin is typically associated with various other biomolecules that influence its physicochemical properties. The two primary methods used are chemical and biological processes, each with its advantages and limitations (Sundar *et al*., 2023).



Chemical Extraction

Chemical extraction is the most commonly used method for chitin isolation due to its efficiency in yielding high-purity chitin. It is particularly effective for large-scale production and ensures consistent product quality. The process involves several key steps:

Demineralization

Demineralization is the first step, aimed at removing calcium carbonate and other inorganic salts present in chitinous materials. This step is crucial, especially when extracting chitin from crustacean shells, which have a high mineral content. Acid treatments are employed, with hydrochloric acid (HCl) and acetic acid being the most commonly used reagents.

Hydrochloric Acid (HCl): This is the preferred choice for demineralization due to its strong acidic nature and cost-effectiveness. Typically, a 1 M HCl solution is used to dissolve calcium carbonate into soluble calcium chloride, which is then removed by washing with water. This approach is particularly effective for crustacean shells, where calcium carbonate can constitute up to 50% of the dry weight (Synowiecki & Al-Khateeb, 2003).

Acetic Acid: In some cases, milder organic acids like acetic acid are used to minimize environmental impact, though they may require longer reaction times or higher concentrations to achieve the same level of demineralization.

The choice of acid and its concentration depends on the source material and the desired purity level. For example, crustacean shells generally require stronger acids due to their high mineral content, whereas fungal sources, which contain less calcium carbonate, can be treated with milder conditions (Kumari *et al*., 2021).

Deproteinization

After demineralization, the next step is deproteinization, which aims to solubilize proteins associated with chitin. This step is crucial for obtaining pure chitin, as residual proteins can affect its physicochemical properties and limit its application in sensitive fields such as biomedical engineering.

Alkaline Solutions: Sodium hydroxide (NaOH) is commonly used due to its strong protein solubilizing capacity. Concentrations ranging from 0.5 M to 2 M NaOH are typically employed at elevated temperatures (60-90°C) for 1-2 hours. This process effectively breaks down peptide bonds and solubilizes proteins, leaving behind the chitin framework (No & Meyers, 1995).

Process Optimization: The concentration, temperature, and duration of NaOH treatment are optimized based on the source material. For example, crustacean shells require higher concentrations and temperatures compared to insect cuticles, which contain less protein.

While effective, this process can partially deacetylate chitin, leading to the formation of chitosan and altering its molecular weight. This is because NaOH also affects the N-acetyl groups present in chitin. Therefore, careful control of reaction conditions is necessary to preserve the desired degree of acetylation (Zhang *et al*., 2020).

Depigmentation and Deodorization

In some cases, pigments such as astaxanthin are removed to enhance the whiteness and purity of chitin. This is particularly important for chitin sourced from shrimp and crab shells, which contain carotenoid pigments.

Organic Solvents: Organic solvents like acetone or ethanol are commonly used for pigment extraction. These solvents dissolve carotenoids and other lipophilic compounds without affecting the chitin structure (Hossin *et al*., 2021).

Oxidizing Agents: Hydrogen peroxide (H_2O_2) is sometimes used as a bleaching agent to remove residual pigments and odours. However, care must be taken to avoid excessive oxidation, which can degrade the chitin polymer. (Maletti et *al*., 2022)

De-pigmentation and deodorization enhance the aesthetic and sensory qualities of chitin, making it more suitable for applications in food technology and cosmetics.

Biological Extraction

Biological extraction methods offer an environmentally sustainable alternative for isolating chitin by utilizing microorganisms and enzymes. Unlike chemical extraction, these methods are more eco-friendly, as they significantly reduce chemical usage and waste generation. Additionally, biological methods preserve the native physicochemical properties of chitin, such as its molecular weight and degree of acetylation, which are often compromised during chemical treatments.

Biological extraction methods can be broadly categorized into fermentation processes, enzymatic extraction, and combined biological processes. Each approach has unique advantages and limitations, influencing the efficiency and quality of the extracted chitin. (Islam *et al.,* 2023)

Fermentation Processes

Fermentation processes utilize microorganisms, particularly lactic acid bacteria, to produce organic acids that aid in chitin extraction. This method is particularly effective for crustacean shells, which contain high amounts of calcium carbonate and protein (Huang *et al.,* 2023).

Lactic Acid Bacteria: Species such as *Lactobacillus spp.* are commonly used due to their ability to produce lactic acid as a metabolic byproduct. The lactic acid lowers the pH of the medium, facilitating the demineralization of calcium carbonate and the deproteinization of chitin simultaneously (Abdel-Rahman *et al*., 2013).

Mechanism of Action: The acidic environment created by lactic acid solubilizes calcium carbonate into soluble calcium lactate, which can be easily removed through washing. Simultaneously, the low pH denatures proteins, making them more accessible to proteolytic enzymes produced by the bacteria (Ta and Nguyen, 2025).

Advantages and Limitations: This method is environmentally friendly, as it avoids the use of harsh chemicals. However, the efficiency of fermentation depends on various factors, including the type of lactic acid bacteria, fermentation time, temperature, and nutrient composition. Additionally, the demineralization process can be slower compared to chemical methods, requiring several days to achieve complete extraction (Younes and Rinaudo, 2015).

Enzymatic Extraction

Enzymatic extraction utilizes specific enzymes to selectively hydrolyze proteins and other non-chitin components without affecting the chitin polymer itself. This method maintains the native structure and molecular weight of chitin, preserving its desirable properties for high-value applications in biomedicine and biotechnology (Islam *et al.,* 2023).

Proteases: These enzymes break down peptide bonds in proteins, effectively solubilizing them. Proteases derived from *Bacillus spp.* and *Aspergillus spp.* are widely used due to their high specificity and activity under mild conditions (Shankar *et al.,* 2021).

Bacillus proteases are particularly effective in alkaline conditions, which enhance protein solubilization while maintaining chitin integrity.

Aspergillus proteases are active in acidic to neutral pH, making them suitable for deproteinization of fungal chitin sources (Jaganathan, 2016).

Chitinases: In some cases, chitinases are used to partially hydrolyze chitin into oligomers, particularly when producing chito-oligosaccharides for biomedical applications. However, this is carefully controlled to avoid complete degradation of chitin. (Poshina *et al.,* 2018)

Deep Eutectic Solvents (DESs): A major focus is on using these "green solvents" to dissolve chitin. DESs are mixtures of compounds that are more effective at dissolving chitin than individual solvents and are considered environmentally friendly (Wang *et al.,* 2022).

Chemical Modification: Another common approach is to alter the chemical structure of chitin to make it water-soluble. This involves creating derivatives, such as sulfonated or phosphorylated chitin, which have improved properties and bioactivity. (Elsabee *et al.,* 2020)

Combined Biological Processes

Combining microbial fermentation with enzymatic treatments enhances the extraction efficiency and reduces processing time. This synergistic approach leverages the advantages of both methods while minimizing their individual limitations.

Synergistic Mechanism: Fermentation with lactic acid bacteria first lowers the pH and partially denatures proteins, making them more accessible to proteolytic enzymes in the subsequent enzymatic step. This combination enhances protein removal and speeds up the extraction process.

Environmental and Economic Benefits: This integrated approach significantly reduces chemical usage and energy consumption compared to chemical methods. It also minimizes wastewater pollution and hazardous byproducts. (Berroci, *et al.,* 2022)

Challenges and Optimization: The precise selection of microorganisms, enzymes, and ideal operating conditions, such as temperature, pH, and fermentation duration, is essential for the success of integrated biological processes. Furthermore, it can be difficult to preserve enzyme stability and microbiological viability during the procedure (Ravi-Kumar, 2000).

Mechanisms of Fungal Disease Management Using Chitin

a. Induction of Host Defence Responses

Chitin acts as a potent elicitor of plant and animal immune responses (Li *et al.,* 2020). When plants or animals detect chitin fragments, they recognize them as a pathogen-associated molecular pattern (PAMP). This recognition triggers immune responses that help combat fungal infections. For instance:

In plants: Chitin oligosaccharides are recognized by pattern recognition receptors (PRRs) on the plant cell surface, such as chitin receptor proteins (e.g., CERK1 in Arabidopsis). This recognition activates signalling pathways that lead to the production of reactive oxygen species (ROS), antimicrobial compounds, and the reinforcement of cell walls.

In animals: Chitin fragments can activate immune cells such as macrophages and dendritic cells, promoting the release of cytokines and the recruitment of immune cells to the site of infection.

b. Disruption of Fungal Cell Wall Integrity

Chitinase enzymes, which degrade chitin into smaller fragments, play a critical role in fungal disease management (Kumar *et al.,* 2018). These enzymes target the fungal cell wall, compromising its structural integrity and ultimately leading to fungal cell death. Many beneficial microorganisms, such as *Trichoderma* spp. and *Bacillus* spp., produce chitinases as part of their biocontrol strategies against pathogenic fungi (Gomaa, 2021).

c. Antimicrobial Properties

Chitin and its derivatives, such as chitosan (a deacetylated form of chitin), exhibit direct antifungal properties. Chitosan, in particular, has been shown to interact with fungal cell membranes, causing leakage of intracellular contents and inhibiting fungal spore germination and hyphal growth.

- Chelate essential nutrients, depriving fungi of resources needed for growth.

Enhancement of Microbial Biocontrol Agents

Chitin serves as a nutrient source for beneficial microorganisms that act as biocontrol agents against fungal pathogens. For example, *Trichoderma* spp. thrive in environments rich in chitin, producing enzymes and secondary metabolites that suppress fungal pathogens. By providing a substrate for these beneficial microbes, chitin indirectly enhances their biocontrol efficacy.

Biofilm Disruption

Fungal biofilms are often resistant to conventional antifungal treatments. Chitosan has demonstrated the ability to disrupt fungal biofilms by interfering with their structural matrix and inhibiting biofilm formation. This property is particularly valuable in medical applications, where biofilm-associated fungal infections are challenging to treat.

Applications in Agriculture

Fungal diseases significantly impact global crop yields, causing economic losses and threatening food security. Chitin-based strategies offer eco-friendly and sustainable solutions for managing these diseases.

Biopesticides

Chitosan-based formulations are used as biopesticides to control fungal pathogens in various crops. These formulations can be applied as foliar sprays, seed coatings, or soil amendments to prevent or reduce fungal infections.

Soil Health Improvement

Incorporating chitin into soil enhances its microbial activity by promoting the growth of chitin-degrading microorganisms. These microbes not only suppress fungal pathogens but also improve soil fertility by recycling nutrients.

Post-Harvest Protection

Chitosan coatings are widely used to extend the shelf life of fruits and vegetables by inhibiting fungal growth during storage and transportation. These edible coatings create a physical barrier against pathogens while maintaining the quality of produce. (Romanazzi, and Moumni, 2022)

Applications in Medicine

Fungal infections in humans, particularly those caused by opportunistic pathogens like *Candida* and *Aspergillus*, are a growing concern due to rising antifungal resistance. Its innate biodegradability, biocompatibility, and non-toxic nature render it an excellent option for medical applications such as wound dressings, drug delivery systems, and antimicrobial agents. The ability of chitin to stimulate immune responses and promote tissue regeneration enhances its therapeutic potential, effectively controlling microbial infections. The derivatives of chitin, such as chitosan, exhibit potent antifungal and antibacterial activities, making them invaluable in preventing hospital-acquired infections. Chitin has the potential to revolutionize biocontrol strategies within healthcare settings, offering sustainable and efficacious medical solutions. (Dave *et al.,* 2021)

Antifungal Therapies

Chitosan-based formulations are being developed as alternative antifungal therapies. Their broad-spectrum activity, low toxicity, and biodegradability make them attractive candidates for treating superficial and systemic fungal infections.

Wound Healing

Chitosan has been incorporated into wound dressings for its antimicrobial properties and ability to promote tissue regeneration. These dressings can help prevent secondary fungal infections in wounds.

Drug Delivery Systems

Chitosan nanoparticles are being explored as carriers for antifungal drugs. These systems improve drug solubility, stability, and targeted delivery, enhancing the efficacy of antifungal treatments while reducing side effects. (Poznanski, *et al.,* 2023)

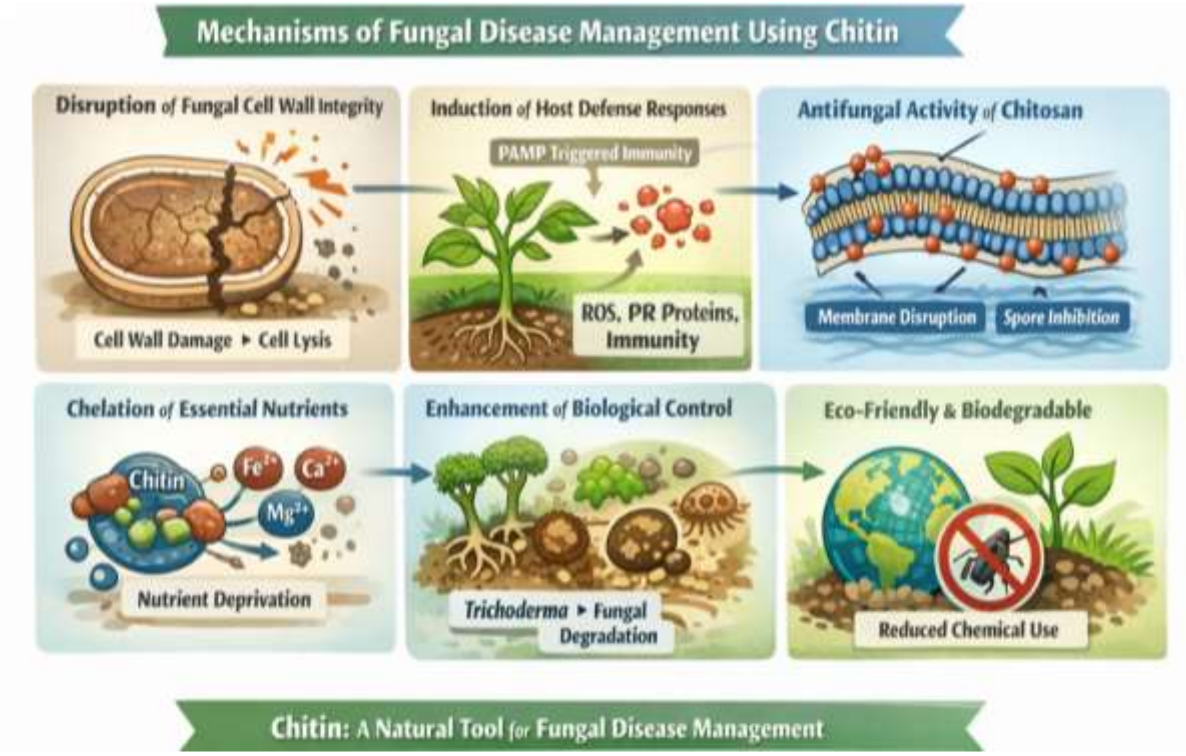


Figure showing how chitin manages fungal diseases through cell wall disruption, immune activation, chitosan antifungal action, nutrient chelation, biological control enhancement, and eco-friendly disease management.

LIMITATIONS AND CHALLENGES

Despite its potential, the use of chitin in fungal disease management faces several challenges:

1. **Limited Solubility of Chitin**: Chitin is insoluble in water and most organic solvents, which can limit its applicability. Chitosan, being more soluble, is often preferred but requires additional processing (Zhang *et al.,* 2024; Bisht *et al.,* 2021).
2. **Cost of Chitin Production**: Extracting chitin from natural sources and converting it into chitosan can be expensive, particularly on a large scale.
3. **Variation in Efficacy**: The effectiveness of chitin-based treatments can vary depending on factors such as pathogen type, environmental conditions, and application methods.
4. **Regulatory Hurdles**: The approval process for biopesticides and medical products derived from chitin can be time-consuming and complex.

5. Future Perspectives

To overcome these challenges and fully harness the potential of chitin in fungal disease management, future research should focus on:

- Developing cost-effective methods for chitin extraction and modification.
- Exploring synergistic combinations of chitin with other biocontrol agents or chemical fungicides.
- Investigating the molecular mechanisms underlying chitin-induced immune responses.
- Conducting field trials and clinical studies to validate the efficacy and safety of chitin-based products.

CONCLUSION

The use of chitin biomolecules in fungal disease management represents a promising approach to addressing the challenges posed by fungal pathogens in agriculture and medicine. Through mechanisms such as immune modulation, antifungal activity, and support for biocontrol agents, chitin offers a sustainable and environmentally friendly alternative to conventional fungicides and antifungal drugs. While there are hurdles to overcome, continued research and innovation hold the potential to unlock new applications for this versatile biomolecule in combating fungal diseases worldwide.

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