Review Article

Chitosan, *Trichoderma* and Zinc Oxide Nanoparticles: A Triad for Plant Growth Promotion and Disease Management

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Abstract | For the sustainable intensification of agriculture, there is an urgent need for innovative approaches with the ability to enhance the productivity of crops without having any negative impacts on the environment or the human health due to the increased use of agrochemicals. This chapter aims to offer an integrated description of the bio-nano synergistic system based on chitosan, *Trichoderma*, and zinc oxide nanoparticles (ZnONPs). Synergy refers to fact that the combined effects of chitosan, *Trichoderma* spp., and ZnO NPs exceed those achieved by the three components individually, leading to e enhanced plant growth promotion and disease management effects. Chitosan; a biodegradable positive biopolymer, acts as a biostimulant, elicitor, plant defense inducer, or nanocarrier for the targeted delivery of agrochemicals. *Trichoderma* spp., the powerful biocontrol agents, act with an enhanced biocompatibility by improving the availability of the biostimulating hormones, and provid microbial bioprotection and bioprospects for the development of biogreen NPs. ZnONPs act with biocompatibility in terms of micronutrient supplementation for their antimicrobial characteristics, with the aid of simultaneous fertilizing-protection. This review comprehensively studies the individual as well as the combined modes of action possible by the biologically interactive chattel, including chitosan, *Trichoderma*, and ZnONPs, through exploring the synergisms that enhance the bioactivities while using reduced amounts of other agrochemicals. On the other hand, this bio-nano synergistic combination offers an innovative advance with the abidance of the principles of nanotechnology, with the development of biologically modified inputs with the aid of the full-volume aspects of bioprospects.

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Introduction

Biostimulants can be an effective tool in alleviating the negative effects of environmental stresses to which plants are exposed, such as drought, salinity, heavy metals and extreme temperatures etc. (Zulfiqar *et al*., 2024). Plant biostimulants refer to biologically derived substances or beneficial microorganisms that enhance plant performance by activating several intrinsic physiological pathways. Rather than acting directly against pests, these inputs improve nutrient assimilation, strengthen stress adaptation mechanisms, and contribute to crop quality under both optimal and adverse environmental conditions (Calvo *et al*., 2014). It is important to note that biostimulants are not the same as pesticides, which act against biotic stresses (Zulfiqar *et al*., 2024), but they primarily address physiological health and resilience rather than acting as direct toxins to pests. Recent data emphasizes that biostimulants can increase yields by 15–20% even under moderate stress, making them indispensable in the face of climate-driven agricultural instability (Zhang *et al*., 2024).

The application of nanotechnology in agriculture enables the design of materials at an ultra-small scale where surface properties dominate behavior. At this dimension, particles exhibit enhanced reactivity, improved interaction with plant tissues, and more efficient delivery of active compounds (Shaikh *et al*., 2021). Nanoformulations can be engineered to release nutrients or act as protective agents gradually, reducing nutrient losses and minimizing environmental dispersion compared to the conventional bulk inputs (Pagano *et al*., 2025). This review focuses on the integration of three distinct but complementary agents that form a potent bio-nano triad which include chitosan, *Trichoderma*, and Zinc Oxide Nanoparticles (ZnONPs). Chitosan is a cationic biopolymer derived from chitin. It acts as an elicitor that triggers the plant's immune system (Induced Systemic Resistance, - ISR) and provides a biodegradable matrix for nanoparticle delivery (Abdallah *et al*., 2020). As for *Trichoderma* spp., they are beneficial fungi; a fungal genus of opportunistic plant symbionts. They are master biocontrol agents that outcompete pathogens for space and nutrients, secrete growth-promoting hormones (auxins), and even act as "factories" for the biological synthesis of NPs (Yao *et al*., 2023). ZnONPs provide essential zinc micronutrients critical for protein synthesis and auxin regulation. Furthermore, they possess inherent antimicrobial properties, creating a physical and chemical barrier against fungal and bacterial pathogens. The synergistic potential lies in their multi-level defense: Chitosan primes the plant from within, *Trichoderma* spp. provide a biological shield at the roots, and ZnONPs offer a dual-purpose nutritional and antimicrobial boost (Mohd Yusof *et al*., 2019). While individual components are well-studied, the "Nano–Phyto–Micro Triad" addresses the critical gap of controlled release and environmental protection of biological agents. This triad concept introduces a hierarchical delivery system where chitosan acts not just as a biostimulant, but as a stabilizing matrix that "envelops" both the biological (fungus) and the chemical (NPs) components, addressing the lack of targeted consistency found in traditional individual applications. However, there are a lot of discussions on how the distinctive properties of nanomaterials may have negative biological impacts as well as be potentially toxic (Ray *et al*., 2009).

The synergistic combination of Chitosan, *Trichoderma*, and ZnONPs enhances crop yield and stress resilience beyond the individual treatments. Research indicates considerable yield increases in crops like garlic, with *Trichoderma* spp. and nano-chitosan elevating garlic bulb weight by up to 22% (Shalaby, 2025). This approach boosts photosynthesis and nutrient absorption, while providing protection against pathogens and drought. The effectiveness of this biostimulant system is dosage-dependent, with recommended encapsulation of ZnONPs in Chitosan for optimal safety and efficacy.

The risk posed by ZnONPs depends on their physicochemical characteristics (such as size, shape, surface charge, and ion release), cultivation settings (including exposure concentration, soil culture, and hydroponics), environmental variables (including temperature, humidity, and light), and the specific plant species involved (Kang *et al*., 2024) . These NPs are predominantly absorbed during the initial phases of plant growth, consequently affecting seedling development and presenting a risk for translocation to consumable parts (Rico *et al*., 2011; Rajput *et al*., 2020). The article reviews the roles of Chitosan, Trichoderma spp., and ZnONPs in agriculture. Key objectives include: (1) Individual benefits where chitosan enhances defense and stress resistance, *Trichoderma* spp. promote root growth and nutrient solubilization, and ZnONPs improve seed germination and photosynthesis; (2) Their synergistic effects that may boost crop productivity and abiotic stress tolerance; (3) Potential for disease control as both chitosan and ZnONPs exhibit antifungal and antibacterial properties, reducing the need for chemical pesticides; (4) Sustainability advantages over conventional agrochemicals in terms of scalability and affordability, especially with cereal crops like wheat.

Chitosan: A versatile biopolymer

Chitosan (CS) is a deacetylated derivative of chitin composed primarily of glucosamine units linked through β-(1→4) bonds. Its biological activity is largely attributed to the presence of positively charged amino groups, which allow interactions with the negatively charged microbial and plant cell surfaces (Román-Doval *et al*., 2023a). Variations in molecular weight and degree of deacetylation remarkably influence its reactivity and functional performance in agricultural systems. It can be obtained from the exoskeletons of crabs, shrimp, and lobsters (primary commercial source), and fungal cell walls. Many fungi, including species used in the triad like *Trichoderma*, naturally contain chitin/chitosan. Moreover, insect exoskeletons include beetles, houseflies, and silkworms contain chitosan also (Pellis *et al*., 2022).

The antimicrobial effectiveness of chitosan is closely associated with its cationic character. Electrostatic interactions between protonated amino groups and negatively charged microbial cell membranes can compromise membrane integrity, alter permeability, and interfere with intracellular metabolic processes. In fungi, the polymer may disrupt cell wall architecture or penetrate the cytoplasm, depending on its molecular characteristics and environmental conditions (Salman and Zedain, 2025). Chitosan has been proved to be effective against bacteria, fungi, and viruses, as it exhibits different inhibitory efficiencies against different microbial species. Due to its large molecular weight, chitosan typically cannot penetrate the cell wall and membrane; thus, its possible antimicrobial actions may involve functioning as a chelator of critical metals, inhibiting nutrition uptake from external sources, and modifying cell permeability (Rabea *et al*., 2003). The mode of antimicrobial action of chitosan is highly dependent on the type of targeted microorganism (Ke *et al*., 2021). Gram-negative bacteria are generally more negatively charged than Gram-positive bacteria because they contain lipopolysaccharides (LPS) in their outer membrane, which are phosphorylated (Raetz *et al*., 2007). The difference in the structure of the bacterial cell wall may be the basis for the higher sensitivity of Gram-negative bacteria compared to Gram-positive bacteria upon treatment with chitosan (Hassan *et al*., 2018).

Chitosan's antifungal properties are mainly attributed to its interaction with the cell wall and cell membrane of fungi. Nevertheless, its effectiveness, *i.e*., its minimum inhibitory concentration (MIC), varies and is influenced by several factors, including its molecular weight (MW), degree of deacetylation (DDA), environmental pH, and species of the fungus (Atai *et al*., 2017; Lopez-Moya *et al*., 2019). Apart from the surface interactions, low-molecular-weight chitosan also penetrates the cell barrier and affects the critical cellular processes, *i.e*., nucleic acid and protein synthesis in the fungal cell (Goy *et al*., 2016; Rohde, 2019). In bacterial systems, the exposure of chitosan has been linked to the suppression of genes that are involved in the growth of the cells and the metabolic pathways, including the biosynthesis of RNA, protein, carbohydrate, amino acid, nucleotide, and lipids (Raafat *et al*., 2008).

In addition to the antimicrobial properties of chitosan, it has been extensively applied as a key component of various seed coating formulations with the aim of providing protection as well as stimulation for the seeds. The coatings are not only effective in prevention of infections caused by fungi and stimulation of germination but are also useful as carriers of other agrochemicals that are released gradually (Godínez-Garrido *et al*., 2021). Moreover, chitosan is an important nitrogen contributor to the soil as it degrades and also improves the soil through the suppression of nematodes *via* the stimulation of chitin-degrading microorganisms that target the eggs of the nematodes or the ammonia produced upon it decomposition (Sharp, 2013).

It was also found that chitosan can induce the production of secondary metabolites in plants, e.g., increasing menthol content in mint plants (Goudarzian *et al*., 2020). The application of chitosan in combination with other natural compounds was found promising in achieving synergism, reducing the dependency on synthetic agrochemicals (Shah and Hashmi, 2020).

Modification of chitosan into NPs, *i.e*., chitosan nanoparticles (CHNPs), overcomes the solubility barriers associated with chitosan (Figure 1). CHNPs can be synthesized using different methods, *i.e*., bottom-up approaches, e.g., polymerization, reverse micelle method, and microemulsion method, and top-down approaches, e.g., milling, high-pressure homogenization, and ultrasonication (Rizeq *et al*., 2019).

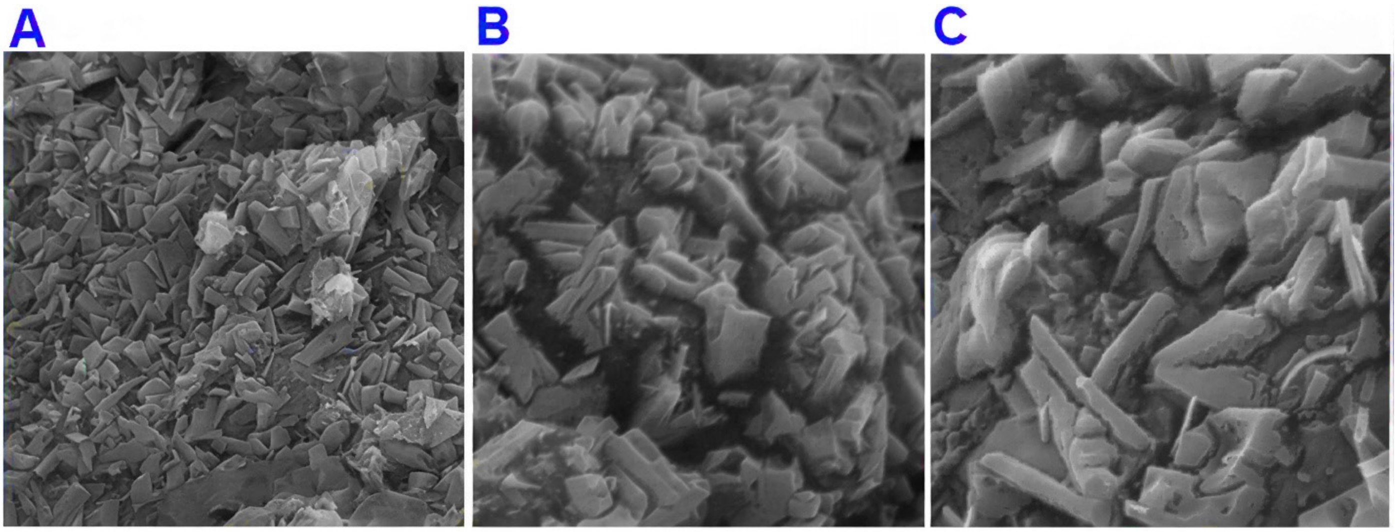

Figure 1: *Scanning electron microscope (SEM) images of chitosan nanoparticles (CHNPs) at different magnifications, (A) 20 µm, (B) 10 µm, and (C) 5 µm.*

Chitosan nanoparticles are effective multifunctional agents in agriculture, functioning as biostimulants and protective tools. Their effectiveness hinges on structural properties, dosage, and environmental conditions. CHNPs outperform bulk chitosan by enhancing plant tissue penetration and stability, and stimulating growth and immunity through signalling pathways. However, plant responses are dose-dependent, where lower doses promote growth, while high doses can inhibit it due to auxin over-accumulation. Environmental factors influence CHNP effectiveness, with yield gains often lower in the field than in laboratory settings. Challenges of scaling CHNPs for agriculture include maintaining size uniformity, stability in various pH environments, complexity of formulation, and regulatory concerns regarding environmental impact (Shah and Hashmi, 2020).

The application of nanochitosan formulations in agriculture not only controls fertilizer, pesticide, and plant growth regulator release but also elicits plant defense responses by activating defense-related enzyme activity (Ingle *et al*., 2022). The delivery systems based on nanochitosan can avoid the overuse of agrochemicals by controlling the release of active ingredients. These delivery systems may also alleviate the challenge of over-pesticide use. (Sharma *et al*., 2019). It has been demonstrated the beneficial impacts of CHNPs and *Trichoderma* spp. on plant growth and productivity. Plants inoculated with *T. harzianum* and treated with CHNPs exhibited enhanced nutritional content, photosynthetic pigments, soluble sugars, and antioxidants, leading to improved growth metrics and augmented garlic yield (Shalaby, 2025). The negative surface charge, small size, and broad surface area of CHNPs promote their absorption, transport, and operating efficiency, increasing the plant nutrient uptake (Sathiyabama and Manikandan, 2021). *Trichoderma* spp. can promote plant growth and development through an auxin-dependent mechanism, as well as auxin mimics, polyphenol content, and antioxidant activity, therefore boosting plant growth, development, and yield simultaneously (Vinale and Sivasithamparam, 2020).

Trichoderma spp.: A multifunctional biocontrol agent

Trichoderma species are among the most versatile and widely utilized filamentous fungi in sustainable agriculture. These soil-borne, opportunistic plant symbionts have evolved from being simple decomposers to sophisticated biostimulants and fungal biocontrol agents (BCAs). Their inclusion in the Chitosan–ZnO triad is pivotal because they provide a "living defense" that adapts to the rhizosphere environment (Guzmán-Guzmán *et al*., 2025). *Trichoderma* spp. are able to survive in harsh conditions, such as extreme ecological environments, including saline marshlands. *Trichoderma* uses biosorption, bioaccumulation, and biotransformation as tolerance mechanisms for Cu, CrVI, Pb, and Cd. On the other hand, there are few investigations on Trichoderma's antioxidant response to microplastic (MP) -induced oxidative stress (Zapata-Sarmiento *et al*., 2025). For effective implementation in biological control measures, selected strains must be able to withstand various environmental stresses, such as changes in temperature, high salinity, and low levels of soil moisture. Furthermore, they must be able to survive and be active even in the presence of fungicides, which are commonly used in management of soils (Omann and Zeilinger, 2010). They are especially appealing for applications in a variety of cropping systems and environmental situations due to their metabolic diversity and ecological adaptability. Additionally, a number of *Trichoderma* strains, including *T. harzianum*, *T. asperellum*, *T. atroviride*, *T. virens*, and *T. longibrachiatum*, have been effectively developed as commercial biofungicides and biofertilizers, accounting for nearly 50–60% of the fungal BCAs (Rush *et al*., 2021). The microscopic observation led to the discovery of a new phenomenon, whereby the hyphae of *T. lignorum* coil around the hyphae of the phytopathogen and penetrate them, subsequently leading to complete dissolution of the host cytoplasm (Dutta *et al*., 2023). Trichoderma spp.s suppress phytopathogens through a coordinated parasitic strategy involving host recognition, directed hyphal growth, and enzymatic degradation of the pathogen's cell wall (Wang *et al*., 2024). This interaction includes physical attachment, coiling around the target hyphae, and secretion of hydrolytic enzymes that ultimately lead to pathogen collapse. These processes are regulated by complex signaling pathways that are activated in response to host-derived cues (Lu *et al*., 2004). By releasing substances that plant receptors will identify as pathogen effectors or by triggering a quicker reaction, *Trichoderma* can enhance Effector-Triggered Immunity (ETI). By generating a range of Microbe-Associated Molecular Patterns (MAMPs), including hydrophobins, expansin-like proteins, secondary metabolites, and enzymes with direct antimicrobial activity, other strains can elicit a stronger immunologic plant response than the pathogen, which means they induce Induced Systemic Resistance (ISR) more than the pathogen induces Systemic Acquired Resistance (SAR) (Topolovec-Pintarić, 2019).

In order to ensure the maintenance of crops yield under low input conditions, the use of biostimulants has been proposed as an alternative that is environmentally safe. These stimulants, which include substances like protein hydrolysates and microorganisms like plant growth-promoting rhizobacteria, are intended to enhance nutrient uptake and productivity (Ventorino *et al*., 2014; du Jardin, 2015).

The *Trichoderma* spp. are well-known to have biocontrol properties; nonetheless, the data about the species mechanisms of promoting plant growth and salt stress tolerance in changing degrees are scarce (De Oliveira *et al*., 2018; Zhang *et al*., 2019). *Trichoderma* spp. are also multi-purpose fungi, which has been shown to promote the growth and grain production in crops through enhancing nutrient absorption and physiological performance. ZnONPs are effective against plant diseases, however at high concentrations (>500 mg/l), they can stop *Trichoderma* from growing. Meanwhile, when combined with ZnONP in soil treatments, *Trichoderma* can evolve mechanisms to tolerate and even lessen the stress caused by ZnONP

Trichoderma spp. have a complex defense mechanism against heavy metal toxicity. This involves the use of extracellular as well as intracellular defense mechanisms. In the former, *Trichoderma* spp. form a physical barrier against heavy metal ions through biosorption to the cell wall as well as extracellular precipitation with organic acids. In the latter, *Trichoderma* spp. induce detoxification of heavy metal ions through thiol-mediated chelation, compartmentalization, and active efflux. In addition to this, *Trichoderma* spp. induce an antioxidant response to combat oxidative stress, which is a byproduct of heavy metal toxicity. This involves the induction of enzymes such as superoxide dismutase and glutathione peroxidase. *Trichoderma* spp. induce morphological changes such as aggregation of mycelia and melanin production (Ramírez-Valdespino and Orrantia-Borunda, 2021).

Meanwhile, the impact of ZnONPs on the growth and development of wheat has been tested. Nanopriming of seeds requires a definite concentration and timing of the NPs. In the case of wheat seeds, the maximum positive effects were found in ZnONPs at 10 mg/l and 18-hour priming time, which produced positive effects on wheat germination, fresh biomass accumulation, seedling vigor, water uptake, and photosynthetic efficiency (Rai-Kalal and Jajoo, 2021).

The current innovative idea involves the use of a combination of heavy-metal tolerant *Trichoderma* isolates with ZnONPs at different concentrations as an effective agricultural approach to improve the germination of wheat and initial seedling development. Such a combined method is an ecofriendly alternative to chemical fertilizers and pesticides (Figure 2). Its integration combines the biostimulating activity of *Trichoderma* spp. *i.e.* root colonization, nutrient

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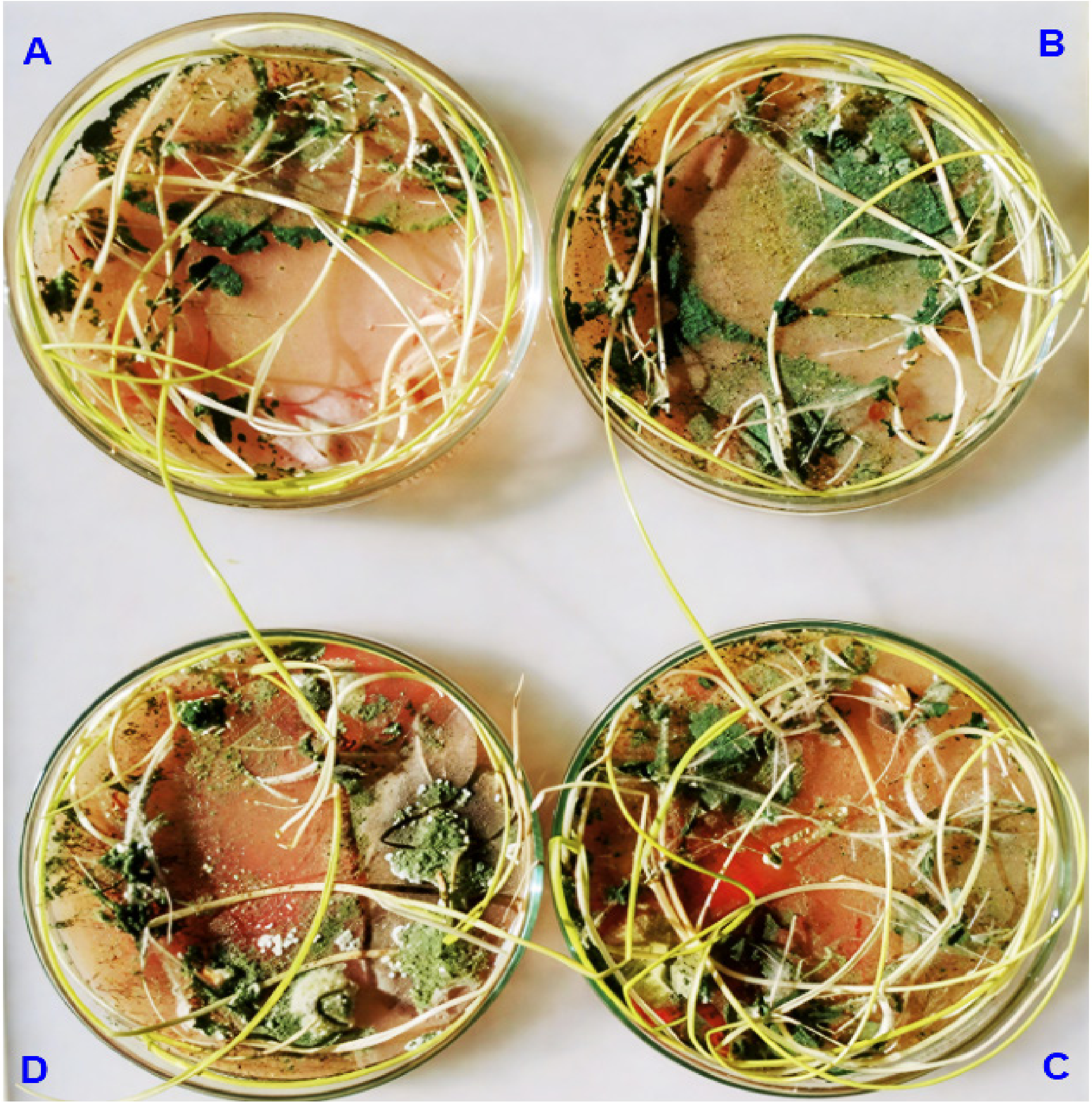

Figure 2: *Trichoderma longibrachiatum isolate combined with three concentrations of ZnO nanoparticles (ZnONPs). (A) Control, (B) 30 μg/ml, (C) 60 μg/ml, and (D) 100 μg/ml applied to wheat seeds. Treatments, particularly at 60 μg/ml, generally exhibited a positive, dose-dependent effect on seed germination and early seedling growth.*

uptake, and growth regulator production, with the targeted delivery and stress-reduction functions of the NPs.

Trichoderma secretes organic acids (*i.e.*, citric, gluconic, and fumaric acids) that lower the rhizosphere pH, effectively "unlocking" these minerals and making them bioavailable to the plant (Song *et al*., 2024). This is in addition tothe synthesis of plant hormones such as auxins, gibberellins, and cytokinins (Illescas *et al*., 2021). A previous study suggested that fungal-plant symbiosis is involved in local and/or systemic signaling, predominantly depending on host genetic factors (Giovannetti *et al*., 2024). *Trichoderma* is highly resilient to metal toxicity, allowing it to be used as a "biological factory." Fungal cell-free extracts can reduce metal salts into NPs (like ZnONPs). These biogenic NPs are often more stable because they are naturally capped with fungal proteins, which reduces their phytotoxicity compared to the chemically synthesized versions. Fungi can resist and detoxify heavy metals (HMs) by several mechanisms, including precipitation, biosorption, complexation, intracellular compartmentation, sequestration chemical modification, and reduced intake, or efflux (Khan *et al*., 2018; Altaf *et al*., 2024). For oxides and salts, the range of chemical composition for mycogenic NPs is much wider, yet most of them remain very poorly studied. Among fungal-mediated oxide NPs, titanium, zinc, iron, and copper oxides have been studied to the greatest extent (Loshchinina *et al*., 2023). Various microbial formulations utilize either solid or liquid carriers to deliver the inoculants effectively. Liquid-based inoculant formulations are cultures of microorganisms that are enriched with water, oil, or polymers. These are the essential components needed to enhance the viscosity of the cell suspensions (Bashan *et al*., 2016; Malusà *et al*., 2016). The major aims of these formulations are to provide a micro-environment conducive to the survival of the microorganisms from rapid loss of viability upon storage, help the introduced microorganisms to compete with the well-adapted indigenous microflora of the soil, and reduce the loss of viable microorganisms due to predation by microfauna upon soil application (Vassilev *et al*., 2020). Several studies have focused on the combination of *Trichoderma* spp. with pesticides for controlling crop disease, where higher control effects have been achieved. The combination of *Trichoderma* spp. and various fungicides could reduce the use of chemical fungicides, which is ecofriendly and may be an important part of the integrated control of *Fusarium* wilt (Li *et al*., 2025).

Zinc oxide nanoparticles (ZnONPs) in agriculture

Zinc oxide nanoparticles are extremely versatile and are considered one of the most widely used candidates in various fields such as medicine, cosmetics, electronics, and textiles (Eren *et al*., 2024). ZnONPs have gained prominence as a multifunctional "nano-fertilizer" and "nano-pesticide." Their ability to deliver essential zinc ions while simultaneously acting as a broad-spectrum antimicrobial agent makes them a cornerstone of the chitosan–*Trichoderma* triad (Javaid *et al*., 2024). Because smaller dimensions are associated with greater surface area, researchers have investigated a variety of synthetic techniques to create ZnONPs with smaller particle sizes. Smaller ZnONPs are extremely sought for a variety of applications due to their increased surface area, which also boosts catalytic activity and quantum efficiency. Direct precipitation, sol-gel, and homogeneous precipitation procedures are traditional methods for creating ZnONPs (Ismail *et al*., 2019; Aghazadeh-Ghomi and Pourabbas, 2021; Wang and Liu, 2024). Furthermore, the creation of ZnO nanostructures with various morphologies, such as nanorods, porous materials, nanospheres, and nanoflowers, has been made possible by sophisticated techniques including supercritical hydrothermal synthesis (Gerbreders *et al*., 2020; Mohan *et al*., 2020).

To improve its synthesis and comprehend its growth dynamics, more research is required because operational parameters have a considerable impact on the creation mechanism of ZnONPs (Sun *et al*., 2024). Green-synthesized ZnONPs and titanium dioxide (TiONPs) are commonly used in photocatalytic applications to break down pesticides, persistent organic dyes, and other resistant contaminants in wastewater when exposed to UV or visible light (Rabiee, 2025). By using ecofriendly reagents, the green synthesis of NPs provides a benign substitute that is more economical and sustainable than the traditional chemical processes (Zaki *et al*., 2022). Green synthesis methodologies further enhance the functionality of ZnONPs by optimizing their particle size, photocatalytic activity, degradation efficiency, biocompatibility, antioxidant properties, and antibacterial potential, particularly in wound healing applications (Singaravelu *et al*., 2025). Their high surface area and superior adsorption properties contribute to their enhanced antimicrobial efficacy (Faisal *et al*., 2021). A promising fungal antagonist for the extracellular production of ZnONPs is *Trichoderma harzianum*. The crystalline structure of ZnONPs is made up of a heterogeneous mixture of rod-, spherical, and hexagon-shaped particles, all of which have remarkably small diameters, according to transmission electron microscopy (TEM) examination (Zaki *et al*., 2021). The size and morphology of NPs are evaluated using different characterization techniques, such as TEM, SEM, polarized optical microscopy (POM), while charge analysis is evaluated by dynamic light scattering (DLS). Each technique complements the others and is chosen based on the specific information needed about the nanoparticles' morphology, structure, and optical properties (Pechyen *et al*., 2024). Table 1 describes how NPs combining with *Trichoderma* spp. enhances plant resilience and disease control: FeONPs boost drought tolerance, AgNPs improve defense against fungal wilt, and ZnONPs suppress soil-borne pathogens, with each pairing leveraging complementary mechanisms to promote growth and stress resistance. An *in vitro* study indicated that the chitosan-Trichoderma-ZnONPs triad had a strong antifungal effect, achieving up to 91% inhibition in addition to rapid synthesis. Field data shows environmental resilience and a 25% increase in garlic yield, although efficacy varies (68-83% disease reduction) due to environmental stresses and ecological interactions, confirming enduring yield advantages (Shalaby, 2025).

Moreover, in a sustainable and efficiency-focused way, CH-ZnONPs) can enhance maize grain production and plant growth performance (Saraswat *et al*., 2024). Subsequently, *Trichoderma* suppresses fungal growth at the root level, while CHZnO-NPs provide foliar/ systemic protection.

Links Researchers

Table 1: *Synergistic effects of Trichoderma in combination with nanoparticles, based on recent researches.*

Nanoparticle	*Trichoderma* species	Synergistic effect and mechanism	Research focus	References
Iron Oxide nanoparticles (FeONPs)	*Trichoderma koningiopsis*	Enhanced Drought Tolerance: The combination boosts antioxidant enzyme activity in plants, reducing oxidative stress more effectively than either component alone. The nanoparticles improve nutrient uptake while *Trichoderma* enhances root growth and stress signaling.	Plant stress mitigation in *Arabidopsis thaliana*.	(Liaquat *et al.*, 2025)
Silver nanoparticles (AgNPs)	*Trichoderma koningiopsis*	Superior Disease Control: The combined application provides a more robust defense against *Fusarium oxysporum* wilt disease. The AgNPs directly damage the pathogen's cell structure, while *Trichoderma* provides a sustained, long-term biocontrol effect through colonizing the root system and competing with the pathogen.	Biocontrol against fungal pathogens in muskmelon.	(Li *et al.*, 2025)
Zinc Oxide nanoparticles (ZnONPs)	*Trichoderma harzianum*	Potent Fungicidal Action: The synergistic effect leads to more effective control of soil-borne pathogens. The ZnONPs disrupt the pathogen's cell membranes, and the *Trichoderma* creates a protective barrier around the plant's roots, suppressing disease and promoting plant growth.	Control of soil-borne pathogens in cotton.	(Zaki *et al.*, 2021)

Antimicrobial activity and micronutrient delivery

Because of their exceptional biological and physicochemical characteristics, CH derivatives have garnered a lot of interest, leading scientists to investigate a variety of CH modification techniques (Rajasekaran and Santra, 2015). To customize its properties for particular applications, these modification techniques range from straightforward one-step reactions to more intricate multi-step procedures, such as direct chemical changes, catalytic or enzymatic transformations, grafting new molecules onto CH chains, and cross-linking under various conditions.

Concerning the chemical reactivity of CH, 2-NH_2 is more reactive than the OH group (Karshalev *et al.*, 2019). Hence, to obtain selective functionalization of the OH group, NH protection by the phthaloyl group was accomplished. On the other hand, OH protection groups like triphenylmethyl, trimethylsilyl and tertiary-butyl dimethylsilyl have also been used to specifically functionalize the NH group, yielding high degrees of substitution (DS) in the products (Lich *et al.*, 2024). CH is soluble in acidic water because of the binding process of H^+ to the N atom of the amino group. However, CH is of a very crystalline nature because of inter- and intramolecular hydrogen bonds. Consequently, it is nearly insoluble in water. CH has also received considerable attention across a broad range of applications, starting with its use as an antimicrobial agent and extending to its use as a drug carrier (Saffarionpour and Diosady, 2025). Quite a few research studies have aimed at unearthing the antimicrobial property of CH over a broad range of microorganisms like bacteria, fungi, yeast, and algae. The main reason for this investigation is its biocompatibility, biodegradability, and non-toxic nature. The antimicrobial property of CH is based on its physicochemical properties, including the degree of deacetylation, structural advantage of having reactive hydroxyl groups on the C-3 and C-6 positions, environmental conditions, and type of the microorganisms (Sabra, 2021). The naturally occurring chitin has low antimicrobial activity because of acetylated repeating units, which limit the accessibility of the amino groups. Moreover, it is a highly hydrophobic molecule because of the presence of strong intra- and intermolecular hydrogen bonding. CH and its derivatives have many uses in agriculture, mostly because they help plants grow, extend their roots and shoots, increase their yield, and support ecofriendly plant growth. The cationic nature of CH derivatives and their ability to interact efficiently with the negatively charged components of the bacterial cell surface led to a bactericidal or bacteriostatic effect. In contrast to this property of acting on bacteria, the chelation ability of CH has made it a very effective antifungal agent (Maurya *et al.*, 2020). It also triggers plant disease protective genes. CH and its derivatives, together with NPs, were also used as foliar sprays to prevent plant diseases, acting as elicitors, plant protection agents, and biostimulants.

For instance, the foliar spray treatment of CH and its NPs on tomatoes acted as an elicitor to increase the enhancement of shoot biomass and flower production, accompanied by a decreased interaction with arbuscular mycorrhizal fungi (AMF). The CH foliar spray treatment alleviated drought stress in Bermudagrass by substantially increasing the quality and content of chlorophyll and relative water, and by reducing the content of electrolyte leakage, malonaldehyde, and hydrogen peroxide (H_2O_2) (Gupta *et al*., 2023). In another experiment, the spray treatment of CH derivatives, including CS-Lactate on *Ocimum basilicum* L. and *Melissa officinalis* L. led to the accumulation of a desirable phenolic compound named rosmarinic acid (RA), accompanied by an increase in shoot biomass. Moreover, by employing CH-SeNPs, they studied the foliar spray on bitter melons, which relieved the salt stress by increasing the activity of antioxidant enzymes and concentration of proline, and maintaining relative water content and K^+, while reducing malonaldehyde and H_2O_2 levels and Na accumulation in plant tissues. Although many studies on CH applications have been going on showing its brilliant prospects for the enhancement of plant growth, disease resistance, and stress tolerance, but there is always a need to define what the best method, dosage, and timing are to work with it most effectively (Dumitriu *et al*., 2021). With regular research, technological innovation, and ecofriendly methodology, CH has the potential to transform the harmless agricultural practices, which may lead to a more sustainable agronomic future. Figure 3 illustrates the dual application of CHNPs through seed treatment and foliar spray and their multifaceted impact on plant development and health. Treated seeds acquire bioactive properties including antimicrobial, antioxidant, and antifungal effects, alongside nutrient delivery and biostimulant functions.

Toxicological considerations and dose optimization

Dimethoate (DMT) is an organophosphate pesticide widely employed in agriculture. However, as a secondary effect, it contaminates water bodies, causing harm to aquatic life. Nanopesticides may be a new and effective approach as an innovative tool in the management of agricultural pests. Considering the above-mentioned points, the current research has put emphasis on the encapsulated DMT in the form of alginate CHNPs (nanoDMT) and its toxicology studies on non-target organisms (EFSA, 2025). This was conducted by testing the characteristics of the NPs using DLS analysis, Nanoparticle Tracking Analysis (NTA) analysis, and Atomic Force Microscopy (AFM) characterization. Additionally, the toxicity of the NPs was compared to DMT, the empty NPs, DMT (NP + DMT), and the commercial DMT (CDMT)

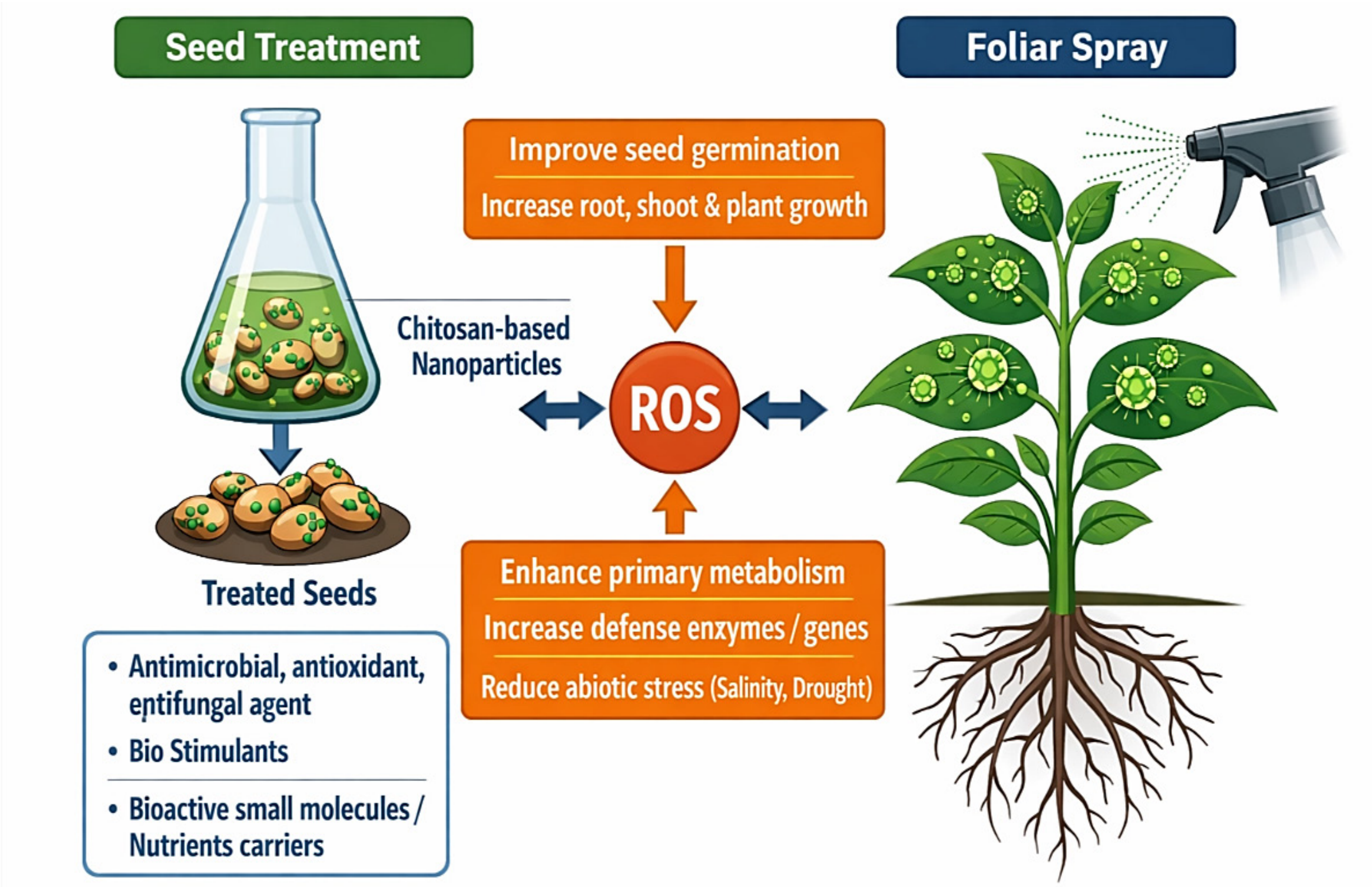

Figure 3: *Mechanistic overview of chitosan-based nanoparticle applications in agriculture via seed treatment and foliar spray, highlighting their role in enhancing plant growth, metabolism, and stress resilience.*

through tests on the embryos and larvae of *Danio rerio* (zebra fish). The factors involved in the distribution of these insecticides in water include, among others, the groundwater flow field, the Organophosphate (OPs) used in agricultural activities, and the season, since the ecological risk of OPs to surface water is higher in summer than in winter due to sunlight (Li *et al*., 2025a). Moreover, a serious risk to non-target organisms such as humans and others like fish may occur due to a leaching process in neighboring aquatic ecosystems in a dose- and time-dependent form. Organophosphate compounds such as dimethoate disrupt neural function primarily by inhibiting acetylcholinesterase activity (Anwar *et al*., 2009). This inhibition results in excessive accumulation of acetylcholine at synaptic junctions, leading to continuous nerve stimulation and subsequent physiological dysfunction. Because acetylcholinesterase is evolutionarily conserved, non-target organisms may also be affected under high exposure conditions (Cheng *et al*., 2025).

Additionally, the neurobehavioral activity of OPs can be induced at very low concentrations that do not profoundly affect the activity of the enzymes. Accordingly, the active mechanisms in these insecticides are not well understood. As OP insecticides exhibit a wide range of toxic mechanisms, some of which are already not well understood, thus a formulation with the potential to lower the amount of the pesticide and its release is much valued in lowering the risks posed by these insecticides to the environment. At the same time, the application of nanotechnology may also be a valuable approach in developing a wide range of novel pesticide formulations with the potential to enhance the safety and efficacy of the pesticide (Sidhu *et al*., 2025). The nanoencapsulation process may be useful for increasing the availability of pesticide in a stable manner through an efficient approach that enhances both solubility and the solubilization process; consequently, the application of the pesticide may yield different results. Drought stress was considerably reduced by low concentrations of ZnONPs (≤ 200 mg/l), which increased root volume by up to 32.0% in the tolerant variety and restored stomatal conductance by 24.6% in the sensitive variety. Conversely, remarkable phytotoxicity occurred at high concentrations (400 mg/l) or with excessive spray treatments (>3 applications), resulting in a 30% reduction in leaf fresh weight and suppressed root growth. ZnO NPs at low dosages increased photosynthetic efficiency by 14.7–49.1%, whereas high doses decreased it by 45.4–66.3% (Wang *et al*., 2026).

Biosafety assessments of ZnONPs require a careful balance between their beneficial and phytotoxic impacts. These effects are highly variable, changing with the plant species, delivery method, and concentration. This variability explains why reported toxicity thresholds differ so widely between studies. ZnONP efficacy follows a biphasic or dose-dependent pattern influenced by species and methodology. While concentrations between 0.1 and 250 µg/ml can be beneficial, toxicity is frequently reported above 20 µg/ml, depending on the study. CHNPs exhibit a similar threshold, shifting from growth-promotion at 30 µg/ml to phytotoxicity at 90 µg/ml (Prajapati *et al*., 2026). Table 2 summarizes some-studies reported concentrations, toxicity points and the primary germination/seedling outcomes for common crops treated with ZnONPs.

Synergistic approaches

Enough agricultural production to meet the demands of the constantly growing global population means the need for intensive agriculture, which also involves adequate plant protection strategies. However, the detrimental effects of overusing chemical plant protectants are also evident on both consumer well-being and the environment. The synergy means the combination of three components yielding a greater result than the sum of their individual impacts. To reduce the adverse effects caused by the overuse of chemicals in agricultural production while pursuing sustainable agriculture, it is time-sensitive to limit chemical usage without compromising efficiency (Qiu *et al*., 2025). Since their method of action is non-specific, copper-based fungicides are also in vogue; there is also no resistance generated in the pathogen against them, and they are also allowed in biologic agriculture. To benefit the bio-economy, copper input should be limited so that it is removed with the harvest. Researchers are investigating combinations with other environmentally benign substances, especially agro-biologics that do not affect the consumers or the environment, in order to maintain potent antifungal effects while using less copper fungicide (Jabborova *et al*., 2025). Chitosan, a naturally occurring substance made from fish waste (chitin), is one intriguing option. It has demonstrated the ability to function in concert with copper-based fungicides. But mixing copper and chitosan isn't easy because they often react to create insoluble precipitates, making product development more difficult.

Despite this difficulty, CH is a sustainable agro-biologic; however, the copper fungicides are still widely used in organic farming because of their efficacy (Iida *et al.*, 2022). A breakthrough was achieved by hydrolyzing CH to a partial breakdown, by producing a mixture of smaller polymers and oligomers that coexist peacefully with copper acetate while still maintaining the antifungal characteristics. This hydrolysate's decreased molecular weight inhibits undesired precipitation, allowing for a 50% dosage reduction in copper without compromising effectiveness (Jin *et al.*, 2024). The agricultural sector is very sensitive to climatic changes; however, in the past few years, the issue of climate change has emerged as a serious concern of our modern civilization. The harmful consequences of climate change were visible in the form of too much rainfall, drought, intense cold weather, heat waves, revival of pests, and the spread of diseases. Consequently, the biological change in the crop's life cycle emerged in the form of decreased production of grains, directly affecting the security of food in the world. Scientists are working on the production of advanced technologies in the agricultural sector to reduce the harmful consequences of climate change and pay close attention to the synthesis of different nanoproducts and the safe dose of these nanoproducts before their use (Kashyap *et al.*, 2023). Additionally, nanofertilizers may hold enormous promise in providing an ecologically safe and economically sound solution to produce crops in the advent of climate change conditions. Nanotechnology-based applications of synthetic fertilizers in agricultural crop production are becoming popular strategies, as have a positive role in increasing crop productivity through improving nutrient use efficiency and reducing the impact of environmental constraints (Kashyap *et al.*, 2022).

Chitosan-ZnO Nanocomposites: Improved stability and functionality

Currently, most pesticides are being used for agricultural crop growth. Most, if not all, pesticides are toxic in nature. Toxic pesticides have polluted ponds, rivers, and soil because of agricultural crop growth. Even pesticide residues found in the final crop harvest are toxic in nature. Pesticides ingested by humans and animals ultimately result in a high death rate. Accordingly, current research by environmental scientists emphasizes the decontamination of pesticides in the environment and in food items using catalytic nanomaterials (Kashyap *et al.*, 2021). Environmental fate processes related to agricultural pesticides are influenced by the physical-chemical properties of the soil system, which are described by multiple factors such as organic matter, sand, silt, clay, water content, and soil pH, in addition to the evaporation, adsorption, decomposition ability, and accessibility for all water resources (Kong *et al.*, 2019). Since pesticide residues alter the microbial biomass in the soil, they act as phytotoxins to the succeeding crops and find their way into the water reservoirs because of the persistence of the compound or the formation of bound residues in the soil. Use of pesticide has been associated with leukemia, lymphoma, and other cancers, as well as Parkinson's disease and congenital abnormalities among farmers, pesticide applicators, and agricultural workers (Kumar *et al.*, 2021). Pollution by pesticide residues affects the biota of the ecosystem. In fact, the accumulation of pesticide residues has a negative effect not only on humans but also on biotic species in the ecosystem. CH, the second most abundant renewable biopolymer after cellulose, has found considerable interest in diverse applications because of its distinct properties like biocompatibility, nontoxicity, and

Table 2: *The balancing beneficial and phytotoxic effects of ZnONPs during seed germination in some crops.*

Species	Beneficial concentration (µg/ml or mg/l)	Phytotoxic threshold (µg/ml or mg/l	Key germination and seedling effects.	References
Maize (*Zea mays*)	80 µg/ml (seed priming improved vigour).	Toxic decline observed above 640–5120 µg/ml in one study.	Increased germination %, shoot/root length, fresh/dry weight and vigor at low dose.	Prajapati *et al.* (2024)
Brassica napus	5 µg/ml (max germination +30%).	≈50 µg/ml caused large root reductions and other toxic effects.	Enhanced germination and rootlet length at 5 µg/ml, where high concentrations reduced rootlet length by ~41%.	Sarkhosh *et al.* (2022)
Camelina sativa	0.1–1 µg/ml (max germination at 0.1 µg/ml; rootlet +15% at 1 µg/ml).	≈50 µg/ml caused severe root length declines (~68%).	Small-dose increases in germination and rootlet length, high doses are strongly inhibitory.	Sarkhosh *et al.* (2022)

antimicrobial properties. However, this compound has limited properties as far as its applications are concerned. Therefore, the use of nanofillers is the best method of enhancing the properties of CH (Kwak *et al*., 2018). In addition, the large number of amino and hydroxyl functional groups in CH molecules enable them to act as effective adsorbents of heavy metal ions and dyes. Within the last few years, the advent of nanotechnology has made nanomaterials promising additives to improve the characteristics of biopolymers.

One of these nanomaterials is ZnONPs, which have gained interest in various sectors worldwide due to their broad-spectrum antimicrobial susceptibility and compatibility, as well as their low cost and simple synthesis process compared to the other metal oxide NPs like FeO and CoONPs (Liu *et al*., 2024). There have been remarkable reports on the use of the CH-ZnO nanocomposites as one of the main materials used in the applications of food packaging materials, coating of the textile material, and the materials used in the dye removal process, attributed to the antimicrobial, antibiofouling property, and photocatalytic ability of the CH-ZnO on the surfaces of the materials as well. The interaction between CH and ZnO occurs when the lone pair of electrons on the nitrogen of CH's amine groups can coordinate with the Zn atoms on the surface of ZnO when CH/ZnO interacts through H of the amine. In the same way, the OH groups' oxygen atoms may likewise interact. The Highest Occupied Molecular Orbital (HOMO) of CH may stabilize as a result of this coordination. Chitosan-coated zinc oxide (CH-ZnO) nanocomposites were prepared using chemical methods to compare their structural and functional properties. Results indicated that these CH-ZnO nanocomposites possess remarkable antibacterial efficacy against various bacterial strains (Liu *et al*., 2022). This finding is true for both Gram-positive and Gram-negative bacteria. Besides, the applicability of the CH-ZnO nanocomposite in various areas exists.

Chitosan-Trichoderma composites: Encapsulation and targeted delivery

Biotic stresses such as bacteria, fungi, viruses, parasites, weeds, and insects may result in yield loss to crops. Among all biotic stresses, fungi are the most destructive to crop yields; therefore, controlling their effects is crucial to minimize damage to crops and prevent financial losses (Manzar *et al*., 2024). The use of chemical protectants against various biotic stresses has been the primary mode of defense for decades, but now there is more emphasis on using ecofriendly materials such as biocontrol agents. Various species of *Trichoderma* have been tapped as potent biocontrol agents, biostimulants, endophytic root colonizers, and plant growth promoters. *Trichoderma* spp. are known to be producers of secondary volatile and non-volatile compounds that have a direct effect on the growth of other fungi (Meshram and Adhikari, 2024). Because of their antagonistic property against a wide range of fungi, *Trichoderma* spp. can be utilized in the control of a range of diseases caused in the root, stem, and leaf parts of the crop plants. The usage of *Trichoderma* spp. in a targeted location through the formulation with a suitable carrier is practiced by different researchers as well as industries for commercial purposes (Hosseyni Moghaddam *et al*., 2020). Because of this approach, the biocontrol agent becomes avoided, as with the formulated product, the controlled biocontrol agent activity is possible, which is feasible and attainable. The use of a formulated product also enables the biocontrol agent to be released in a sustained manner. The carriers are usually talc, bentonite clay, or kaolin for powder formulation and vegetable oils and mineral oils for liquid formulation. The natural polymer CH, with its antimicrobial activity, which is utilized as a plant defense inducer, growth promoter, and in the management of plant pathogens, can be utilized as a better carrier material for biocontrol agents' delivery system (Muscolo *et al*., 2013).

The interaction of *Trichoderma* spp. with metal nanoparticles (MNPs) differs substantially, reflecting the beneficial and adverse roles of MNPs. For instance, high doses of MNPs such as ZnO may cause damage to the *Trichoderma* cell wall by an oxidative stress. In contrast, low doses of MNPs may promote mycosynthesis, with *Trichoderma* proteins acting as stabilizers of MNPs and limiting their toxicity. However, the challenge of maintaining fungal viability and controlling agglomeration of MNPs persists. For example, chemical surfactants used to stabilize ZnONPs may inhibit fungal growth and the pH conditions for stability may not favor the fungus. Therefore, various encapsulation techniques such as CH and alginate matrices are being used to store *Trichoderma* spp. until the target site is reached (Shalaby 2025).

Various application techniques of *Trichoderma* spp.

include seed treatment, soil application, foliage spray, etc. Seed treatment assists the seed in overcoming the effects of soil ecology, and also protects it against seed-borne and soil-borne pathogens and premature emergence, and aids in getting healthy plants. CH was found to be an antifungal agent against post-harvest horticultural diseases in plants caused by fungal pathogens such as *Fusarium sulphureum*, *Alternaria alternata*, *Aspergillus niger*, and *Rhizopus oryzae*. One of the methodologies includes the simultaneous application of *T. harzianum* and CH for the control of sap stain pathogens. Chitin/chitosan-containing formulations have been found to counteract various phytopathogens effectively, and increase the plant growth remarkably (Olivares *et al*., 2017). A chitin-supplemented peat formulation of *Bacillus subtilis* AF1 was found to be extremely effective in advancing groundnut and pigeon pea growth. A combination of CH and *Trichoderma* spp. can improve disease control and increase vigor of the plants. Besides the antimicrobial property of CH, its film-forming ability can provide physical protection even for the seeds (Patel *et al*., 2022). However, the permeable nature of this film enables the required vapor and gas exchange in the seed prerequisite for healthy germination and better establishment of the plant. This attributed to the fact that the film formation on the surfaces of seeds by CH provides protection from the stresses, which is already overcome by the combination of a plasticizer and a cross-linker, because the film formation resulted in the production of a brittle, non-uniform layer on the seeds rather than an uninterrupted film of CH (Qiu *et al*., 2024).

Trichoderma-ZnONPs: Enhanced antagonistic activity and plant colonization

Nanotechnology has a wide range of applications and is a transformative field in several areas like drug delivery systems, cosmeceutics, industrial applications, and even agricultural applications. The emerging technology utilizes specific features and properties of matter at the nanoscale, ranging from 1 to 100 nm. One of the considerable areas of nanotechnology is the design and synthesis of NPss. The NPs can be synthesized by different biological and chemical/ physical methods (Rathod *et al*., 2024). The use of a physical and chemical method in NPs synthesis is not very advantageous because it requires dangerous chemicals and more aggressive process conditions like pressure, energy, and heat. The process of generating NPs in this way has several disadvantages and releases of harmful by-products. The use of the biological mode or green nanotechnology in the synthesis of NPs seems more reasonable and viable, because the process and chemicals used are ecofriendly and not harmful. Green synthesis of the NPs may be performed using different biological objects such as bacteria, fungi, cyanobacterial systems, actinomycetes, and plants (Sahariah *et al*., 2018).

Metal-based NPs have received the greatest interest among all the other NP forms, which are being used in agriculture because they possess the property of interacting with the biological objects at the molecular level. ZnO NPs possess antifungal and antimicrobial properties and therefore demonstrate enhanced efficacy as nanopesticides compared to the commonly used pesticides. Additionally, ZnONPs exhibit the ability to interact with the plant pathogens at the cell membranes, resulting in the disruption of their structure. They also possess the capacity to enter the fungal and bacterial cell walls, causing the disruption of the membranes of the bacteria and fungi (Suwanchaikasem *et al*., 2024). This damage is attributed to the Zn^{2+}ions leaking into the environment as the ZnONPs take time to dissolve. Such zinc ions can inhibit the critical enzyme activity, protein biosynthesis, and DNA biosynthesis. They are harmful to the microbial cells. The ZnONPs exhibit direct antifungal activity. In plants, however, ZnONPs induce systemic resistance too. Mustard (*Brassica juncea*) is a major oilseed crop used for producing a quality edible oil with high protein-rich meals. It possesses improved nutrition standards and food security worldwide. Production-wise, it is the third oilseed crop globally after soybean oil and palm oil. However, foliar diseases contribute substantially to reduced mustard yield (Tyagi *et al*., 2024). Their magnitude of reduction largely depends on the severity, environment, and methods used for production. Among the major foliar diseases that infect mustard crops are their infection by *Albugo candida* (white rust), *Erysiphe cruciferarum* (powdery mildew), *Peronospora parasitica* (downy mildew), and *Alternaria brassicae* (Alternaria blight). Among all the foliar diseases infecting mustard, Alternaria blight is an exceptionally damaging disease. Its severity may bring about potential yield loss ranging from 32 to 57%. Traditional approaches, which include the use of fungicides and resistant varieties, can be used for managing Alternaria blight in mustard, but these approaches face challenges in terms of manageable longevity and environmental sustainability (Van Der Voort *et al*., 2016). There is, therefore, an increasing need for more innovative or alternative approaches for managing fungal diseases. Nanotechnology has emerged as an area of sustainable agricultural practices for managing plant diseases. ZnONPs displayed antifungal activity against *Alternaria alternata* on tomato plants due to the destruction of fungal cell membranes and subsequent death of the fungal cells. ZnONPs expresed strong antifungicidal activity against *Fusarium oxysporum* in tomatoes and cucumbers due to the inhibition of the germination of fungal spores and the mycelial growth. There are also negative aspects of using NPs in agriculture, especially MNPs, which put limitations on their use in management of plants (Emannuel Oliveira Vieira *et al*., 2024). High concentrations of metal/metal oxide NPs, such as AgNPs and ZnONPs, can cause oxidative stress due to an increase in ROS production, which results in lipid peroxidation, DNA damage, and loss of cellular architecture. A higher concentration of ZnONPs (500 µg/ml) may lead to phytotoxicity relative to lower concentrations, which may negatively impact the morphological properties such as a decrease in the length of roots and shoot of the wheat seedlings. Genotoxicity, such as chromosomal aberrations, micronuclei, and mitotic index reduction, has been reported in plants such as *Vicia faba* treated with AgNPs. Moreover, with increasing NPs accumulation within the environment, there are concerns regarding potential exposure through uptake by the food chain and danger to higher organisms, such as human beings (Wang *et al*., 2023). Despite such challenges, optimal concentrations of NPs may be of great significance in managing plant diseases through their antimicrobial properties used to combat plant pathogens.

Table 3 indicates the evolution of agricultural biotechnology, which has recorded a move from individual treatments towards integrated systems. Three key aspects are discussed in this table, namely: (1) the foundational role of individual treatments, which, in the past, used CH as a growth stimulant, *Trichoderma* spp. as biological control agents, and ZnONPs for nutrient supply, with safety concerns related to their used concentrations; (2) the synergistic advantages of dual combinations, in which the combination of treatments, such as CH and *Trichoderma*, offers several advantages such as increased bioavailability and biocontrol; and (3) the conceptual triad system, where all three components are integrated into a multifunctional bionano-carrier system, where CH serves as a scaffold, ZnONPs provide antimicrobial properties, and *Trichoderma* enhances plant resilience in an attempt to overcome the limitations observed in the traditional agriculture.

Table 3: *Synergistic integration of chitosan, Trichoderma spp., and ZnO NPs: a roadmap from individual treatments to bionano triad systems.*

Approach	Components	Reported effects	Crop/system	Key findings	References
Individual treatment	Chitosan	Enhanced plant growth, activation of plant defense responses, and antimicrobial activity.	Tomato, wheat	Improves seed germination and increases antioxidant enzyme activity	El Hadrami *et al*. (2010)
Individual treatment	*Trichoderma* spp.	Biocontrol against pathogens, improved nutrient uptake, and induction of ISR.	Various crops	Promotes root development and systemic resistance.	Harman *et al*. (2004)
Individual treatment	ZnO nanoparticles	Improved micronutrient availability, and enhanced photosynthesis and growth.	Wheat, maize	Low concentrations stimulate growth, and high concentrations may cause phytotoxicity.	Dimkpa and Bindraban (2016)
Dual combination	Chitosan + *Trichoderma*	Enhanced disease resistance and plant growth.	Tomato	Increased defense enzyme activity and pathogen suppression.	Elsharkawy *et al*. (2021)
Dual combination	Chitosan + ZnO nanoparticles	Improved antimicrobial activity and nutrient delivery.	Various crops	Nanocomposites show stronger antifungal activity than individual components.	Kumar *et al*. (2022)
Dual combination	*Trichoderma* + ZnO nanoparticles	Improved growth promotion and pathogen suppression	Rice, tomato	Enhanced root growth and disease resistance.	Raliya *et al*., (2017)
Proposed triad system	Chitosan + *Trichoderma* + ZnO nanoparticles	Potential synergistic effects in plant growth promotion, nutrient delivery, and disease management.	Conceptual framework	Integration of biopolymer carrier, beneficial microorganisms, and nanonutrient delivery.	ND*

*Where; * indicates not detected.*

Applications of plant–microbiome nanotechnology

The amalgamation of plant microbiome science and nanotechnology provides an entirely novel approach for achieving sustainable agricultural systems and improving plant health. In this study, the focus will be on exploring the diversity, composition, and functions of plant microbiome science that encompass rhizosphere, phyllosphere, and endosphere microbiota (Wei *et al*., 2021). In this context, it is important to present their vital functions in plant development, path defense mechanisms, and ecological adaptation. In terms of functional aspects of microbiome science, it has been upgraded with multiscale approaches provided by multi-omics technology that enable comprehensive observations of host-microbe interactions and mutualism. At the same time, nanotechnology has been transforming agricultural practices into a completely improved sustainable agriculture system, substantially enhancing nutrient potential, stress tolerance, and e microbial diversity present in the soil (Wu *et al*., 2016). Nanotechnology has a remarkable impact on microbial functions, plant metabolism, and soil properties, all of which contribute to increased crop production. About nanomaterials integration with useful microorganisms, there has been vast potential for high-precision agriculture that has been made available *via* artificial intelligence and the internet (Wu *et al*., 2015). The reviews point out new concepts in the engineering of microbiomes using NPs, with most being in conceptual stages or pilot studies, thereby requiring more validation. The main areas include precision microbiome engineering with desirable effects on plants, targeted delivery systems for modulation, and regulatory and ecological issues with long-term use of NPs in agriculture. The application of all these concepts on various crops and soils is also subject to validation.

Influence on nanoparticles rhizosphere microbial balance

The plant microbiome is composed of several microorganisms, including bacteria, fungi, viruses, protists, and archaea, which work collectively to improve crop productivity through their intricate coactions with plants in the environment. The beneficial microorganisms are found in the phyllosphere, rhizosphere, and endosphere. They exhibit numerous functionalities to their respective plants, including plant growth promotion, biopotential assistance, chemical prevention, biocycling, disease protection, and stress resilience. Bacteria and fungi are major microorganisms in plants compared to the other microorganisms in the soil ecosystems (Afzal *et al*., 2025). On the other hand, archaea, protists, nematodes, and algae play a crucial role in assisting plant growth. The rhizosphere soil contains high concentrations of beneficial plant microbial life. Plant roots serve as facilitators, allowing beneficial microbial life to inhabit them and develop into endophytes. In addition, microorganisms play major roles in rhizosphere soil, where they produce antibiotics to control plant development by regulating soil-borne diseases to develop immunity within plants due to poor environmental conditions. Relative abundance of the main groups of bacteria in the rhizosphere and total soil is comparable, and they are mostly from the Proteobacteria phylum (Kafle and Joshi, 2025). The abundance of the bacterial groups, however, differs greatly in the rhizosphere, endosphere, and phyllosphere. The plant endosphere is dominated by Firmicutes and Proteobacteria. The phyllosphere is mostly dominated by bacterial other groups such as Bacteroidetes, Actinomycetes, Proteobacteria, and Firmicutes. The main fungi that infect both the underground and aerial plant parts are mostly from the Basidiomycota and Ascomycota phyla. The bacteria in plant and soil ecosystems also have complex friendships with other organisms (Cruz *et al*., 2024). The types of relations are defined as communality, parasitism, and mutualism. Parasitism leads to the incidence of many plant diseases, which in turn result in the loss of crop growth in agricultural plants. The community association of soil bacteria (and archaea) and plant groups, especially legumes, leads to the process of nitrogen fixation. It also provides the mechanism of biofertilizers, which enhance the growth of plants. The other element of the usage of the microbiome in botany is in the form of biopest control, where the negative microorganisms are utilized in the synthesis of a pesticide that is related to the enhancement of the disease control in plants. There have been appreciable efforts in the past two decades in the modification of the plant microbiome for efficient plant functions. Recently, innovative, efficient, and ecofriendly nanotechnologies have been introduced that have considerable applications in botany, including modulation of the plant microbiome (Zhang *et al*., 2025). These nanotechnologies have the potential to overcome the limitations of the conventional microbiome modification methods, which are non-specific in their ability to achieve targeted modifications, may damage microbial diversity, and lack consistency in their results. The aim of nanobiotechnological mivroorganisms trait interaction modification is to develop an efficient-oriented, ecologically sustainable agricultural approach with the promising impact of enhanced plant development performance due to the reduced usage of chemical fertilizers and pesticides, thereby mitigating the environmental impact of agricultural practices and, by extension, their effects on human health (Wu *et al*., 2019). Figure 4 describes the nanotechnology toolbox used for agrochemicals and soil microbiome modulation. The central part of the figure shows a growing plant and root system, symbolizing the rhizosphere and the interface where NPs and soil microorganisms meet. The four groups of nanoscale agricultural materials that are generally categorized and illustrated in the figure can be briefly described. The biodegradable polymeric NPs family, including Polycaprolactone nanoparticles (PCLNPs), Polylactic acid nanoparticles (PLANPs), Poly (lactic-co-glycolic acid) nanoparticles (PLGANPs), Polyethylene glycol nanoparticles (PEGNPs), and others categorized as chitosan nanoparticles (CHNPs) play an important role in their biodegradability and application for controlled release of nutrient substances, pesticides, and microbial biostimulants. The family of carbon nanomaterials, including Carbon Nanotubes (CNTs), Graphene Oxide Nanoparticles (GONPs), Carbon Nanodots (CNDs), and Multi-Walled Carbon Nanotubes (MCNTs) can be represented by their high surface area and potential for binding

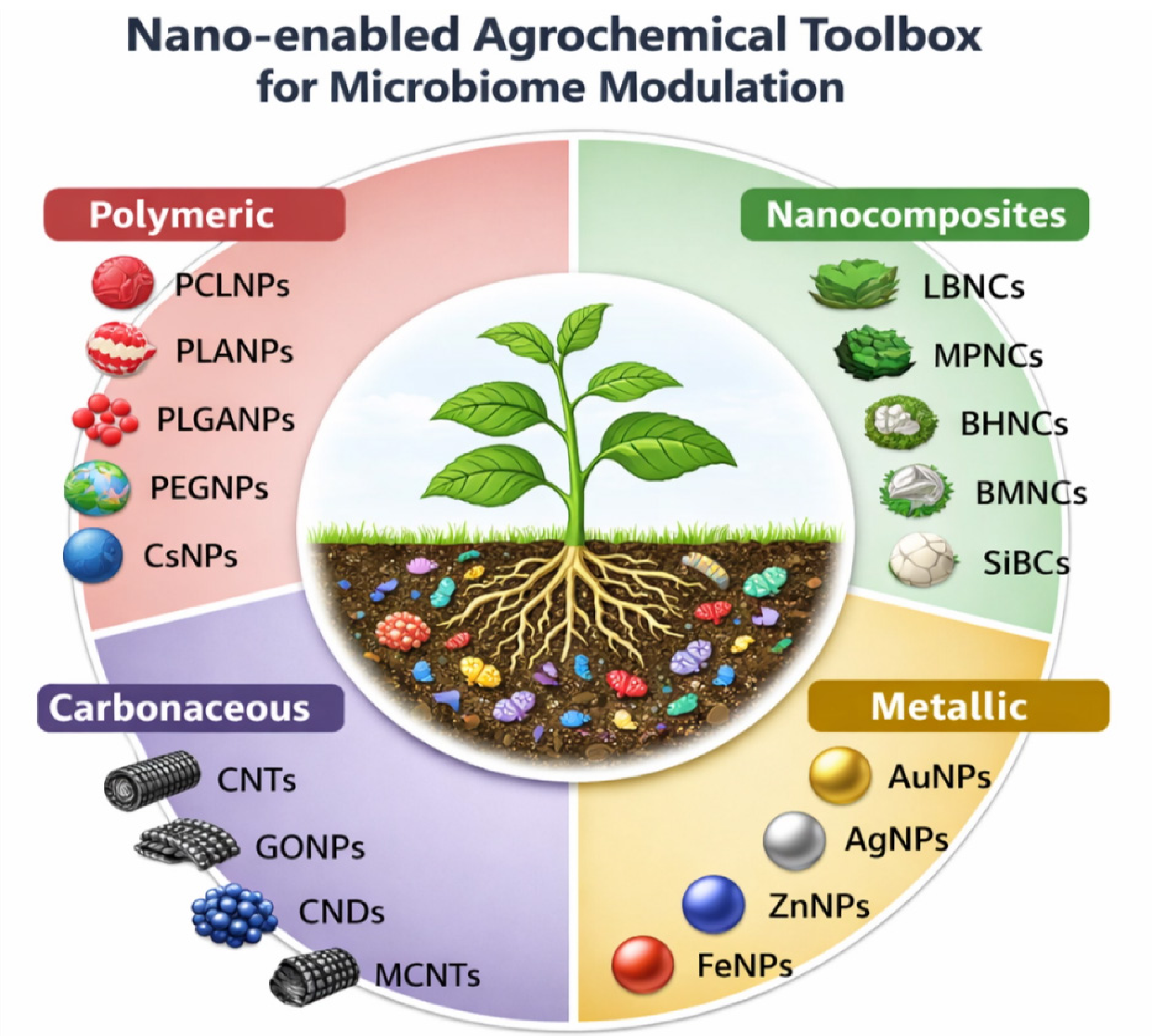

Figure 4: *Nano-enabled agrochemical toolbox for plant microbiome modulation. This scheme summarizes four major classes of nanomaterial polymeric, carbonaceous, metallic, nanocomposites, and their representative types.*

to microbes, which makes them more biologically active and efficient for microbe communication and nutrient provision. The metallic NPs family of AuNPs, AgNPs, ZnNPs, and others represented by FeNPs offers outstanding antimicrobial potential, micronutrient supply, and stress-protection properties but necessitates considerations regarding toxicity. This class of nanocomposites includes diverse biopolymeric nanomaterials with multi-component features. Each group together implies nanotechnology strategies for soil microbiome regulation and more productive crop growth. An available study reported positive pairwise interactions between the components, but few studies have investigated the combined effect of the three components, with reported outcomes indicating improved disease management and nutrient uptake by the plants (Abd-Elsalam *et al*., 2024). Seed priming with *Trichoderma* spp. and foliar spraying with ZnONPs was reported to reduce the incidence of Fusarium wilt and promote the growth, nutrient content, and activity of defensive enzymes in cherry tomato (Chaudhary *et al*., 2021). CH-NPs with *T. viride* or mycorrhizal fungi reduced the incidence of the late wilt disease and improved the growth of maize seedlings. Among the treatments, the combined effect of CH and mycorrhizal fungi was the best in controlling the various disease in maize, although the combined effect of CH and *T. viride* also exhibited positive outcomes (Kumar *et al*., 2025). ZnO-CH composites have a major effect on the composition of the bacterial community and rhizosphere by offering protection against root diseases, increasing the bioavailability of nutrients, and improving the plant growth. It was reported that nanomaterials could act synergistically with beneficial microorganisms to enhance plant growth but also highlighted the risks involved with the ecotoxicological and nontarget effects of nanomaterials on the microorganisms (El-Gazzar *et al*., 2018).

Modulation of root exudates and nutrient cycling

The hidden heroes of grassland ecosystems are plant roots, which subtly support vital functions like soil health, carbon storage, and nutrient recycling. The active regulation of carbon (C) and nutrient fluxes by roots in response to changing land use and climate circumstances is examined in this study (Wang *et al*., 2023). Synthesis of root biology, soil chemistry, and ecosystem science reveals how root morphology and chemical exudates dictate carbon sequestration and nutrient cycling within shifting environmental contexts (Uddin *et al*., 2021).

Furthermore, we review climate change-induced alterations, including changes in precipitation patterns, temperature extremes, and elevated atmospheric CO, together with effects on rooting-mediated C and nutrient fluxes. Plant roots are considered the focal point of grassland ecosystems in terms of C and nutrient cycling, as they participate in a vast array of processes that influence the health of the grassland ecosystem. This section introduces the essential plant root characteristics and processes involved in the sequestering of C and nutrients as well as stabilization of the soil (Shah *et al*., 2023). Root morphology and architecture describe the characteristics of the plant root systems, such as size, density, and geometry, including root extension, density, size, and depth pattern. The importance of root system characteristics has been widely acknowledged to define the plant efficiency in exploring soil-water and soil-nutrient resources. Root morphology affects the plant exploration and the plant support ability. Root morphology, architecture, and exudations are unique and supplementary components to belowground ecological process mediation. The role of fine roots with a high surface area index increases soil-nutrient and soil-water uptake. The effect of laterally extending plant root architecture enhances subsoil explorations of plant roots. Moreover, root exudates containing organic acids, amino acids, or secondary metabolites/exsubstances exudated through the plant roots act as stimulants of microbial processes such as enzymatic activities, which play a pivotal role in N-mineralization, P-solubilization, and efficiency of microbial C utilization. Grassland plants have diverse root patterns that can be classified into two major types: deep-rooted and shallow-rooted plants, depending upon their natural adaptability to the diverse environments (Sathe *et al*., 2021). The root patterns of the different plant species demonstrate the diversity of root characteristics of plants such as their root depth, which ranges from deep-rooted trees to shallow-rooted grasses. Every individual plant root system is of utmost importance in terms of C storage, nutrient acquisition, and soil stability (Saberi-Rise and Moradi-Pour, 2020). A complete knowledge of the respective differences among the diverse plant species is a prerequisite to comprehend the effects of the land use changes and the climatic conditions on C storage in grasslands.

Plant growth promotion and nutrient uptake

Land use changes, including grazing, agriculture, and deforestation, have played a considerable role in affecting plant root processes and functions related to carbon sequestration, nutrient cycling, and soil erosion in grasslands (Nguyen *et al*., 2023). The plant roots are highly sensitive to any level of environmental stress, and changes in root structure, biomass, or function have a direct effect on the ecosystem process and the function (Pour *et al*., 2021). However, sustainable land use approaches such as crop rotation and cover crops have been explored to improve the underground root biomass, soil organic carbon content, and amendment nutrients. The land use approaches have direct effects on plant root structure, architecture, and exudate secretion, which are important indicators of key ecosystem services such as soil quality, water regulation, carbon sequestration, and erosion control (Moon *et al*., 2017). Grazing is one of the major land use types that takes place in grassland ecosystems. Grazing intensity ranges from beneficial to damaging depending on the magnitude of root development. When root development is negatively affected by overgrazing, there is a notable decrease in root biomass, particularly in the topsoil layer, which contains the root distribution of most grass species. This is due to proximity to the resources such as water. When there is a decrease in root biomass, it leads to reduced C sequestration rates within the ecosystems. When herbivores consume the aboveground biomass, there is a decrease in the root/shoot ratio, which eventually results in reduced C inputs into the soil (Kalembasa and Jenkinson, 1973). Figure 5 presents a conceptual framework showing how management practices and disturbances regulate the plant biomass allocation and the soil carbon–nutrient dynamics. Fertilization influences plant community attributes, particularly species composition and richness, which directly affect the aboveground (shoot) biomass production.

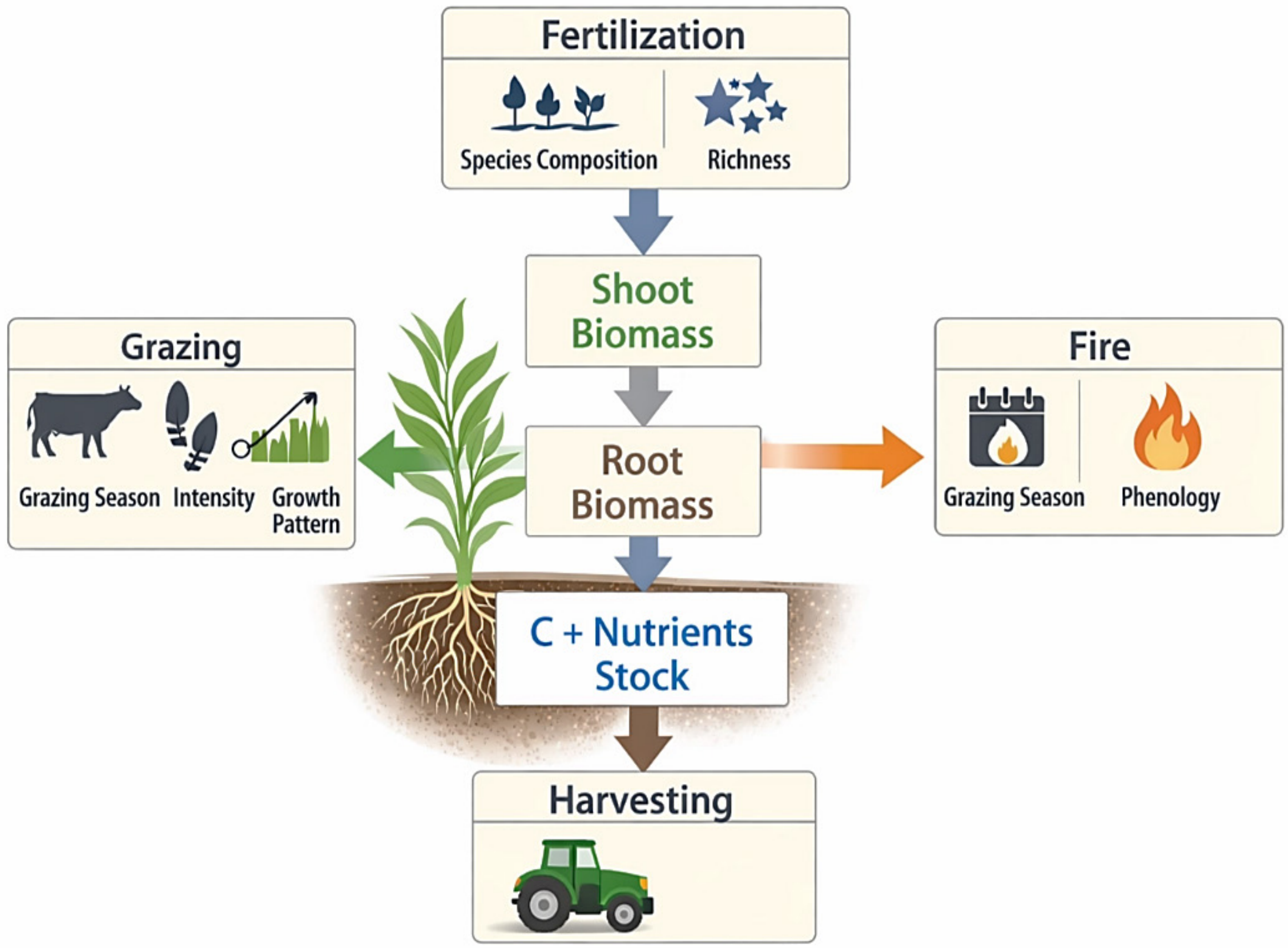

Figure 5: *The effects of fertilization, grazing, fire, and harvesting on plant biomass allocation, soil carbon (C), and nutrient stocks. Management practices and disturbances regulate shoot and root biomass, which together control the underground C and nutrient dynamics.*

In conclusion, nanomicrobiome engineering is defined as the development of tools that facilitate nano-enabled delivery of microbial or nutrient functionalities without compromising the native rhizosphere. The key aspects to be considered include physicochemistry, formulation, dosage, and biosafety. The behavior of NPs in the soil is essential in determining their effects on the microbiome and the plants. The assessment of safe dosage is also essential to avoid toxicity to non-target microorganisms. The carrier and encapsulation techniques should be effective to ensure the long shelf life of the delivered nutrients. Consistency in manufacturing is essential in determining biological effects, with regulatory assessments being integral in determining long-term environmental effects on soil health.

Phytotoxicity, biosafety, and environmental impact

Assessing nanoparticle's toxicity on plants and soil health

Because of the antioxidant, biocompatibility, bio-adhesive, and free radical-scavenging ability, CH provides a high potential for being a phytoprotector, cytoprotector, and genoprotector against the toxic pesticide/agrochemicals. Agrochemicals have long been extensively renowned for their phytotoxic, cytotoxic, and genotoxic properties against the plant, cell, and DNA, respectively. Accordingly, for their application, the toxic pesticide or agrochemical could possibly be incorporated or loaded inside the spherical nanocapsule matrix of CH, which in turn create a protective barrier or shell and avoid or prevent the direct cellular or DNA damage caused by the toxic pesticide or agrochemical (Li *et al.*, 2025b).

To verify this hypothesis, greenhouse and nursery experiments have been performed for the analysis of NP's phytotoxicity, while 3-[5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assays have been carried out under the *in vitro* cell viability study for the analysis of cytotoxicity. In addition, the comet assay, which is a powerful tool for detecting damage to DNA strands was also employed. Several plant development parameters, for instance, total fresh weight, leaf area, root biomass, and overall leaf mass of Chilli plants, were found to have gained remarkable improvements following CH-NPs application. The treated plants were found to perform better compared to their untreated counterparts, and this was performed in a specific order (Inobeme *et al.*, 2025). The robust growth of barley and wheat seedlings treated with CH-NPs provided additional evidence for these conclusions. Research on chitosan-thiamine NPs, for example, showed that these NPs are not hazardous to the plant cells because they considerably reduced cell death in *F. oxysporum*-infected roots compared to the untreated plant samples. The outcomes were even more remarkable when chitosan-alginate and chitosan-tripolyphosphate were used as nanocarriers for herbicides such as imazapic and imazapyr (Maruyama *et al.*, 2016). Compared to utilizing free herbicides, encapsulating these herbicides completely avoid cell damage and achieve a 100% reduction in modification, according to previous cytotoxicity studies conducted on onion roots (Amaç and Liman, 2021).

A comet experiment demonstrated that, from the standpoint of an overall safety, the hamster ovary cells exposed to NPs suffered from less DNA damage than those exposed to free herbicides. The biocompatibility of chitosan-tripolyphosphate herbicide NPs was demonstrated by the remarkable preservation of DNA integrity observed in cells treated with them, which was the same as in the untreated control group (O'Farrell, 2025).

Chitosan hexaconazole NPs also proved capable of diminishing cytotoxic effect values of the Vero cell line compared to the conventional hexaconazole formulation as well as the free hexaconazole (Román-Doval *et al.*, 2023b). Moreover, the assessment showed that chitosan carbendazim NPs had lower cytotoxic effects on the human lens epithelial cell line compared to the free carbendazim. CH-NPs have been shown in the present study to be non-toxic to the human lens epithelial cells. Additionally, the half lethal concentration ($LC_{50)}$ value was considerably raised by encapsulating the pesticide in chitosan-poly (2-dimethylamino-ethyl methacrylate) microcapsules, even though both the conventional and the free pyraclostrobin displayed acute toxicity in the zebrafish within 24 hours of exposure (Xu *et al.*, 2018). This change suggests a considerable decrease in toxicity, offering compelling proof of chitosan's function as a potent cytoprotective, genoprotective, and phytoprotective agent against the dangerous pesticides (Huq *et al.*, 2022). CH delivery technologies such as nanocapsules and nanoemulsions, reduce the cellular toxicity of the pharmaceuticals like triclabendazole in the intestinal epithelium. Beyond increased absorption, these nanoformulations provide a higher safety profile than the standard ones. These findings are corroborated by tests on the HeLa cell line, which showed that chitosan-quinapyramine sulfate NPs had a substantially lower genotoxicity and cytotoxicity than the free drug (Vallim *et al*., 2022).

Biodegradability and safe delivery systems

The farming sector has generally been dependent on substantial usage of agrochemicals such as fertilizers, pesticides, and herbicides. However, the use of such agrochemicals is not favored for future generations due to safety concerns related to health, microbial flora, soil mineral nutrients, and the development of resistance in pathogens. Furthermore, usage of various biopolymers for smart delivery of bioactive components has gained popularity in recent trends to reduce soil and water contamination by excessive usage and nonspecific delivery of agrochemicals (Malerba and Cerana, 2016). Moreover, in the past few decades, CH nanomaterials have gained enhanced interest in the farming sector for smart delivery of bioactive compounds because of their biodegradability, biocompatibility, and non-toxicity.

To design NPs for specific usage purposes, such nanomaterials have the promising capacity for slow release of agrochemicals, targeted delivery of bioactive components, and optimized usage of plant nutrients with reduced exposure to the environment. Application of CH nanomaterials for the encapsulation of fertilizers, pesticides, herbicides, micronutrients, and genetic materials is possible for sustainable farming (Malerba and Cerana, 2020). The CH degradation process is enzyme/chemical-induced *in vitro* and *in vivo* and can be defined as degradation of the polymer into smaller units known as monomers (*i.e.*, D-glucosamine, N-acetyl-glucosamine). The rate of degradation of CH is very important upon applying it as a drug delivery carrier and in tissue engineering applications. The level of degradation and molecular weight are considered fundamental in modulating the CH degradation rates. Moreover, any chemical modification of CH will play a major role in affecting its degradation rates. Among the enzymes mostly used as catalysts in CH degradation are the lysozymes (Khan *et al*., 2022).

Regulatory and biosafety aspects in field application

Many studies are greatly concerned about minimizing the detrimental effects of agrochemicals on both the individual well-being and the environment due to the unavoidable use of these chemicals in crop management. The danger to a person's health is determined by the agrochemicals' toxicity and the exposure to the application/residue in food and drinking water (Hundleby *et al*., 2018). In addition, conventional agrochemicals have major negative effects on the environment as they dissipate and leach. Direct handling and chronic exposure to these materials within the agricultural sector may lead to systemic issues, including respiratory distress, nausea, and skin irritation (Beeckman and Rüdelsheim, 2020). As discussed in the earlier sections, the encapsulation of agrochemicals within the CH NPs provides a controlled release mechanism, which in turn assists in lowering the wastage and leaching of agrochemicals. Further, the longer circulation time and higher efficacy of the chitosan-based agronanochemicals also diminish the requirement for the active dosage of agrochemicals, thus minimizing the problems of run-offs of agrochemicals in the environment (Deepansha *et al*., 2025).

Gaining knowledge about how soil microbial populations react to pesticide treatments might help one better understand how the soil ecosystem cycles the nutrients. Enzyme activity and changes in the composition and quantity of many microbial species, including bacteria, fungi, actinomycetes, protozoa, algae, and yeasts, are examples of these processes. Researchers have examined how chitosan-based nanoformulations and traditional agrochemicals affect soil microbial populations in this regard (Zhang *et al*., 2020). A study has indicated that the use of free herbicides such as paraquat can adversely impact the microbial ecosystems and drastically lower the soil enzyme activity. Herbicides enclosed in CH NPs, on the other hand, typically have no effect on the microbial activity and exhibit reactions that are similar to those of the untreated control soils (Tahir and Zahra, 2025). The enhancement effect on the soil microbial population after the 7th day of treatment results from the use of chitosan-alginate-herbicide NPs compared to the free herbicides (*i.e.*, imazapic and imazapyr). The obtained findings indicate the capability provided by the CH nanocarriers in reducing the negative side effects associated with the use of the toxic agrochemicals (O'Farrell, 2025).

Conclusions and Recommendations

The combination of chitosan, *Trichoderma* spp., and ZnO nanoparticles is a powerful and progressive approach for dealing with the closely interlinked issues of crop yields, health, and sustainability. As shown in this review, every element in this trio provides unique but complementary functions in relation to crop management and sustainability. While chitosan can serve as a biodegradable biostimulant, defense elicitor, or smart nanocarrier, *Trichoderma* spp. can act as dynamic biologic controllers, biostimulators, and/or microbiome modifiers. Additionally, ZnONPs act as dual-purpose agents: they provide the necessary micronutrients and offer high biocidal activity against the pathogens. Taken together, the synergistic effects of these components far surpass those of any individual component. Evidence discussed emphasizes the potential of this bionano system to decrease the dependence on the conventional agrochemicals by the controlled release and the targeted delivery, with increased efficacy upon lower application rates. Such a reduction is more important for reducing environmental contamination, preserving soil microbial diversity, and lessening phytotoxicity or other non-target effects. Advances in green synthesis, nanoformulation, and microbiome-aware use further demonstrate that deployment under sustainable and climate-resilient agriculture is highly feasible. Despite the encouraging results, a responsible deployment is necessary. Optimization of dosage, time, and method of applications together with detailed long-term environmental fate, biosafety evaluation, and compliance with regulatory requirements is crucial to ensure safe field deployment. Future studies are recommended to focus on field-level validation, multi-omics-driven insight into plant-microbe-nanomaterial interactions, and integration of precision agriculture tools to tailor the intervention with the crop and the agroecosystem specificity. Overall, the trio of chitosan, *Trichoderma*, and ZnONPs presents a paradigm shift that will usher in the next-generation era of agriculture through the integeration of biological innovations with the field of nanotechnology.

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Novelty Statement

The review presents a novel triadic system, which incorporates chitosan, heavy metal tolerant *Trichoderma* spp., and ZnONPs in a synergistic combination system to promote growth and control of disease in plants. This idea contrasts with the conventional methods of utilizing the extreme chemical fertilizers and pesticides, as it focuses on plant's growth biostimulation in the form of root colonization, nutrient uptake, and growth regulator production caused by *Trichoderma* spp., with added stimulation and stress alleviation abilities of the ZnONPs, and secured by the protective and elicitor attributes of the CH NPs. This suggested integration not only leads to better germination of wheat, soybean, or other crops and early seedling development but also creates a new design of ecofriendly crop management, which provides an alternative to the chemical input in contemporary farming.

Author's Contribution

FKA, RME, MGF, AMG: Conceptualization, Data curation, investigation, supervision, validation roles, writing original draft, and writing review and editing.

Generative AI and AI-assisted technology statement
The authors attest that no artificial intelligence (AI) or AI-assisted technology was used during editing of this work.

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