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The Impacts of Chitosan on Plant Root Systems and Its Potential to be Used for Controlling Fungal Diseases in Agriculture

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Abstract

Chitosan is a natural elicitor, used for stimulating plant growth and inducing plant defense. However, due to difficulty in monitoring root growth and activity, the effects of chitosan treatment on plant root systems have been less studied as compared to plant shoot parts that include leaves, seeds, and fruits. This results in an indefinite outcome of the benefits of chitosan on plant roots. Therefore, this review aims to evaluate the effects of chitosan treatment on root growth and defense responses based on current evidence. Interestingly, many studies have demonstrated that chitosan can induce plant root defense systems, yet conversely inhibiting root growth. The effects were most clearly observed from studies using liquid or solid media as substrates, while the results from the studies using soil were inconclusive and require additional investigation to observe the effects of environmental factors. In addition, root chitosan treatment showed variable effects on shoot growth, where low chitosan concentrations tend to promote shoot growth, but high chitosan concentrations may affect shoot development. Additionally, this review discusses the potential methods of chitosan application onto plant roots. Water insolubility of chitosan is likely a major issue for root treatment. Chitosan can be dissolved in acids, but this could induce acidity stress in plant roots. Modified versions of chitosan, such as chitosan nanoparticles, carboxylated chitosan, and graft chitosan copolymers have been developed to improve solubility and functionality. Chitosan nanoparticles can also be used to encapsulate other biocontrol agents to augment biological effects on plant defense. In conclusion, root chitosan treatment could help to promote plant defense and prevent root infections, abating the uses of chemical fungicides in agriculture. However, further research is required to monitor the impact of root chitosan treatment on long-term plant growth in order to gain multifaceted information to maximize the effectiveness of root chitosan application.

Keywords Chitosan nanoparticles · Elicitor · Growth-defense tradeoff · Plant defense · Plant growth · Root growth inhibition

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Introduction

Chitosan is a natural polymer, composed of β-1,4-linked *N*-acetyl-D-glucosamine (*N*-GlcNAc) and D-glucosamine subunits. It is used in a range of applications to benefit humanity. In biomedical and pharmaceutical applications, it is used as a drug carrier, vaccine adjuvant, wound dressing material, and cartilage and bone tissue engineering scaffold (Muxika et al. 2017). Chitosan is also utilized in the food and agricultural industries owing to its antimicrobial, antifungal, and plant defense-eliciting properties (Pongprayoon et al. 2022; Shahbaz et al. 2022).

Chitin is another natural polysaccharide made of β-1,4-linked *N*-GlcNAc subunits. It is the primary source of chitosan for both natural production and industrial synthesis (Fig. 1). Chitin is one of the most abundant polymers in the world and predominantly found in fungal cell walls and exoskeletons of insects and crustaceans, such as scorpions, spiders, beetles, crayfishes, shrimps, and crabs. In contrast, chitosan is only found in the cell walls of certain fungal species and to a lower degree than chitin. Naturally, chitosan is synthesized from chitin by deacetylation using specific chitin deacetylase enzymes (Fig. 1). Chitosan can be extracted from fungal cell walls of *Mucor* spp. and *Rhizopus* spp. in the Mucoromycota phylum, *Candida* spp. and *Saccharomyces* spp. in the Ascomycota, or *Pleurotus* spp. and *Lentinus* spp. in the Basidiomycota (Ghormade et al. 2017). For mass production, chitosan is converted from chitin via chemical reactions using a strong base and high heat (Younes and Rinaudo 2015). Since shrimp and crab shells are common by-products from the seafood industry, chitin and chitosan are considered as readily accessible and affordable natural resources (Younes and Rinaudo 2015).

Different techniques of chitosan preparation affect physicochemical properties of chitosan end-products, such as size, viscosity, crystallinity, and degree of deacetylation, which could result in different biological activities and suit different applications (Brasselet et al. 2019; Román-Doval et al. 2023). Sources of chitin and chitosan materials, types of chemicals used in demineralization and deacetylation steps along with chemical concentrations, and temperatures and times are important controlling factors in chitosan preparation (Román-Doval et al. 2023; Younes and Rinaudo 2015). Sizes of chitosan can be divided into low- (< 150 kDa), medium- (150–700 kDa), and high molecular weight (> 700 kDa) (Boamah et al. 2023). Viscosity and solubility of chitosan are related to the size of chitosan—the larger or longer polymers, the higher viscosity, and the lower solubility (Aranaz et al. 2021). Crystallinity determines other physiological properties, such as porosity, water-absorption, and moisture-retention properties (Román-Doval et al. 2023). Degree of deacetylation is a key qualification of chitosan end-products. The high degree of deacetylation such as 80% deacetylation means that 80% of acetyl groups

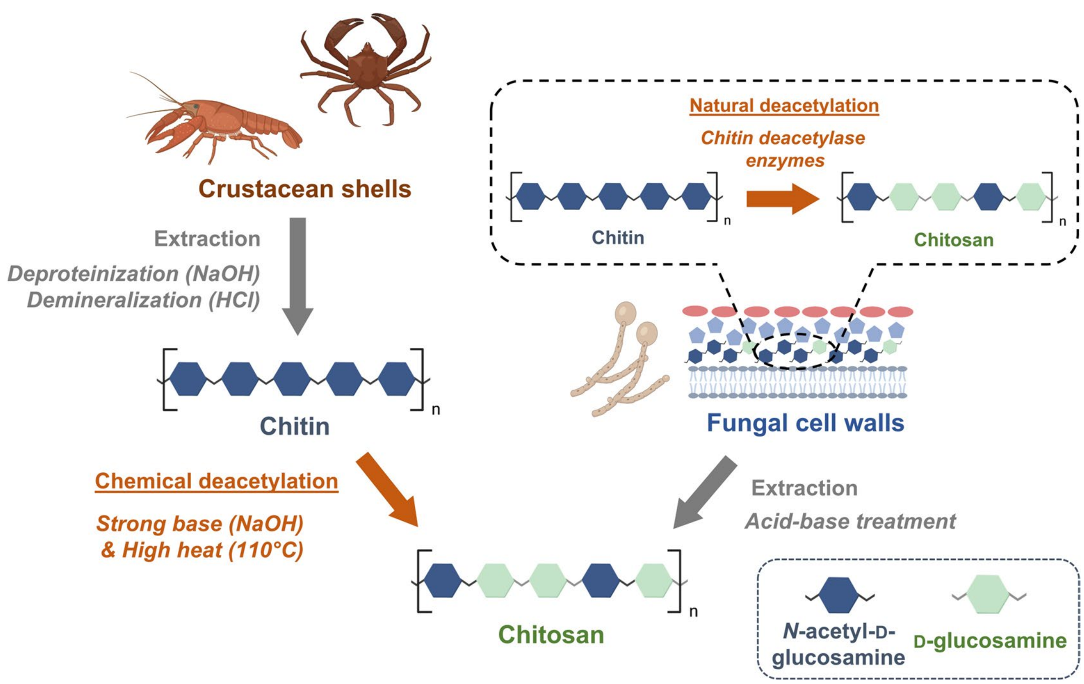

Fig. 1 Schematic diagram of chitosan preparation from two main natural resources, including crustacean shells and fungal cell walls. This figure was created with the assistance of Biorender.com

in *N*-GlcNAc monomers of long-chain chitosan are deacetylated. Higher degrees of deacetylation associate with increasing chitosan-like properties, including advancement of some physicochemical properties of chitosan, such as increased solubility in acidic environment (Brasselet et al. 2019). In the market, chitosan products come in a wide range of sizes, crystallinity, and degree of deacetylation. These parameters have a significant impact on chitosan biological properties and its applications. For example, low-molecular weight chitosan can be used as a plant elicitor, due to its enhanced solubility and higher antimicrobial activities but high-molecular weight chitosan is usually developed into film and used as external protectants, such as for seed or fruit coating (Boamah et al. 2023; Román-Doval et al. 2023). Nonetheless, chitosan either derived from fungal cell walls or processed from marine organisms could yield similar end products if they have identical physicochemical properties. The details of chitosan preparations, formulations, chemical and biological properties, and potential applications are well articulated in many review articles (Aranaz et al. 2021; Elieh-Ali-Komi and Hamblin 2016; Jimenez-Gomez and Cecilia 2020; Khan and Alamry 2021; Morin-Crini et al. 2019; Román-Doval et al. 2023; Younes and Rinaudo 2015; Yu et al. 2021).

In agricultural application, chitosan has been studied and used to promote plant growth and induce plant defense. Under biotic stress conditions, chitosan treatment has been shown to enhance plant resistance against pest infestations and pathogen infections (Fan et al. 2023). Several methods can be applied to perform chitosan treatment: for example, seed soaking before sowing, foliar spraying during plant growth, or fruit coating after harvesting (Riseh et al. 2022). The evidence supporting the protective roles of chitosan on aboveground plant tissues is well established (Chakraborty et al. 2020; Divya and Jisha 2017; Malerba and Cerana 2016, 2018; Pichyangkura and Chadchawan 2015; Riseh et al. 2022; Sharif et al. 2018; Stasińska-Jakubas and Hawrylak-Nowak 2022; Yu et al. 2021). However, the protective effects of chitosan have been investigated less on plant root systems. This is potentially due to the challenges of working with belowground materials, which are hard to observe and sample from (Lopez-Moya et al. 2019; Suwanchaikasem et al. 2022). As a consequence, studying plant roots requires effort, strategic planning and specialized tools to facilitate root visualization, treatment, and analysis.

The root system is an indispensable part of plants. It functions to anchor plants in place and absorb nutrients and water. Roots also form a complex biosystem with a myriad of surrounding microorganisms and organic substances underground, called the rhizosphere, making roots subject to regular fluctuations of environmental factors (York et al. 2016). Any changes of soil microbes, nutrients, moisture, pH, and temperature could affect plant growth (de la Fuente Cantó et al. 2020). Hence, understanding root activities and interactions with surrounding environments is fundamental knowledge, especially relevant in agriculture to support plant growth and improve crop yield. Common practices including supplying fertilizers, plowing soil, removing weeds, adding beneficial microbes and applying chemical pesticides, herbicides, and fungicides are routinely carried out to support root functions and to promote overall plant growth (Hakim et al. 2021; Watt et al. 2006). However, overusing pesticides and fungicides can be hazardous to humans, plants, and environments (Sharma et al. 2019; Tsalidis 2022). To limit or avoid the use of chemicals, natural elicitors such as chitosan could be an alternative platform for pest and disease management. Therefore, understanding plant root responses to chitosan treatment is essential to underpin the practical implementation of substituting chemical pesticides and fungicides with natural elicitors in agriculture. Furthermore, although the effects of chitosan have been well demonstrated on plant shoot tissues, the effects of chitosan on plant root systems might be different to what appears to the shoots due to a variety of environmental factors attributed to plant shoot and root tissue exposures.

This review aimed to assess the current knowledge from research that applied chitosan treatment on plant roots and monitored overall plant growth and defense responses. A literature search was conducted using three online databases, ScienceDirect, Web of Science and Wiley Library, using the basic search function most recently in April 2023. Search terms included "chitosan," "root," "growth," and "defense," where "chitosan" and "root" were fixed terms, while "growth" and "defense" were variable terms. The search was performed multiple times using AND as a Boolean operator between each term for all combinations. Date and year of publication were not restricted. The search results were filtered by article type, where research article was selected, while other forms, such as review article, book, book chapter, and proceeding abstract, were excluded. The number of returned hits varied from 90 to 4,263 publications (Table S1).

Subsequently, the results were sorted by "relevance" mode, where the most relevant articles were first listed. Starting from the first article, the method section of each article was checked to ensure that chitosan was applied onto plant roots, and overall plant growth and defense were monitored. The studies that applied chitosan on shoot tissues, such as foliar spray and seed soaking, were excluded, even though root responses were monitored. Likewise, the studies that applied chitosan on plant roots but did not examine plant physiological or biological responses were excluded. For returned lists with over 200 articles, once twenty articles in a row were found to be unrelated to the search criteria, the checks were stopped, and the rest of the articles were omitted. Duplicate articles across different searches were also removed. Finally, the shortlist was cross-checked with any review articles that contained sections summarizing the effects of chitosan on plant roots. Research publications related to the topic but absent from the shortlist were additionally included. In total, the number of research articles identified and included in this review was 24. Fifteen articles performed root chitosan treatment in in vitro or tissue culture settings. Nine articles reported the effects of root chitosan treatment under soil conditions. Most of the articles reported either growth parameters or defense mechanisms. Only five articles monitored both growth and defense responses concurrently in the same study.

This review is organized into eight sections. In "Impact of Root Chitosan Treatment on Shoot Growth Varies Upon Chitosan Dose and Treatment Factors" and "Root Development is Inhibited by Chitosan Treatment" sections summarize the effects of root chitosan treatment on shoot and root growth, respectively. In "Root Defense is Induced by Chitosan Treatment" section discusses the effects of chitosan on biological and biochemical plant defense. In "Growth-Defense Tradeoff is a Result of Root Chitosan Treatment" section integrates information from sections "Root Development is Inhibited by Chitosan Treatment" and "Root Defense is Induced by Chitosan Treatment" to surmise that root growth-defense tradeoff could be a consequence of root chitosan treatment. In "Methods to Apply Chitosan Onto Plant Root Systems" section articulates possible pathways of chitosan application onto plant roots. In "Combination of Chitosan Treatment with Other Methods for Fungal Disease Control" section describes the potential of chitosan when used in combination with other techniques to manage diseases. In "Conclusion and Perspective" section concludes the key messages and suggests further studies to advance the effectiveness of chitosan application. This review provides inclusive information regarding the effects of chitosan on plant root systems, which could encourage the use of chitosan in plant disease management schemes.

Impact of Root Chitosan Treatment on Shoot Growth Varies Upon Chitosan Dose and Treatment Factors

Chitosan is well demonstrated to promote plant growth when applied as a foliar spray. For example, periodically spraying chitosan (0.0125–0.1% w/v) on strawberry leaves for two months prior to flowering significantly increased plant height, leaf size, individual, and total fruit weights. Upon harvest, fruit biomass was increased by 29–42% (Rahman et al. 2018). Spraying 0.1% chitosan on one-month-old tomato leaves significantly promoted the number of flowers and fruits per plant by 14% and 77%, respectively. Total fruit fresh weights per plant increased by 2.45 times (Sathiyabama et al. 2014). The results from other studies showing shoot growth promotion according to chitosan foliar spray are well collated and discussed in review articles (Chakraborty et al. 2020; Divya and Jisha 2017; Malerba and Cerana 2018; Pichyangkura and Chadchawan 2015; Sharif et al. 2018; Stasińska-Jakubas and Hawrylak-Nowak 2022). In contrast, root chitosan treatment has not been consistently shown to promote shoot growth. As a biostimulant, chitosan was also expected to stimulate overall plant growth and could be one way of improving crop yield when applying to plant roots by mixing with soil or adding into hydroponic solution (Asghari-Zakaria et al. 2009; Ohta et al. 2001). However, the results in the literature show variable outcomes of root chitosan treatment on shoot growth, which is different from the results of the direct application of chitosan on shoot tissues.

Studies have demonstrated the positive effect of root chitosan treatment toward shoot growth (Fig. 2). For example, in chili (*Capsicum annuum*), after 30 days grown in soil amended with 1% w/w high-molecular weight chitosan, plant height, and leaf area were increased by 2.5–3 times and the number of fruit and fruit weight per plant were approximately 10 times increased. Treatments with low- and medium-molecular weight chitosan also conferred similar shoot growth promoting results (Chookhongkha et al. 2012). In prairie gentian (*Eustoma grandiflorum*), after 10 weeks grown in soil supplied with 1% chitosan, leaf size was increased by approximately 40–60% and shoot fresh and dry weight were promoted by approximately 5–6 times (Ohta et al. 2001). Moreover, supplying soil with both fertilizer and 1% chitosan showed a synergistic effect, where all shoot growth parameters were significantly higher than the single treatments of either chitosan or fertilizer alone (Ohta et al. 2000). The shoot growth-promoting effect of root chitosan treatment was also observed in other ornamental plants, where total shoot fresh weights of lobelia (*Lobelia erinus*), gloxinia (*Sinningia speciosa*), and Persian violet (*Exacum affine*) were increased by 52, 26, and 9 times, respectively, after approximately 6–13 weeks of soil amendment with 1% chitosan as compared to the normal condition of untreated fertilized soil (Ohta et al. 2004). In tomato (*Solanum lycopersicum*), plants were grown in soil drenched with 1% w/v chitosan solution (dissolved in 1% acetic acid) and compared to the control plants, grown in soil drenched with pure water or neutralized acetic acid. After 1–2 months, plant height, shoot fresh and dry weight of chitosan-treated plants were 1.5–2 higher than the controls (Algam et al. 2010). These results suggest that root chitosan treatment has potential to promote shoot growth upon soil cultivation.

However, several studies have shown conflicting results (Fig. 2). For example, in lettuce (*Lactuca sativa*), after 35 days grown in the soil amended with chitosan, the number of leaves per plant, leaf area, shoot fresh, and dry

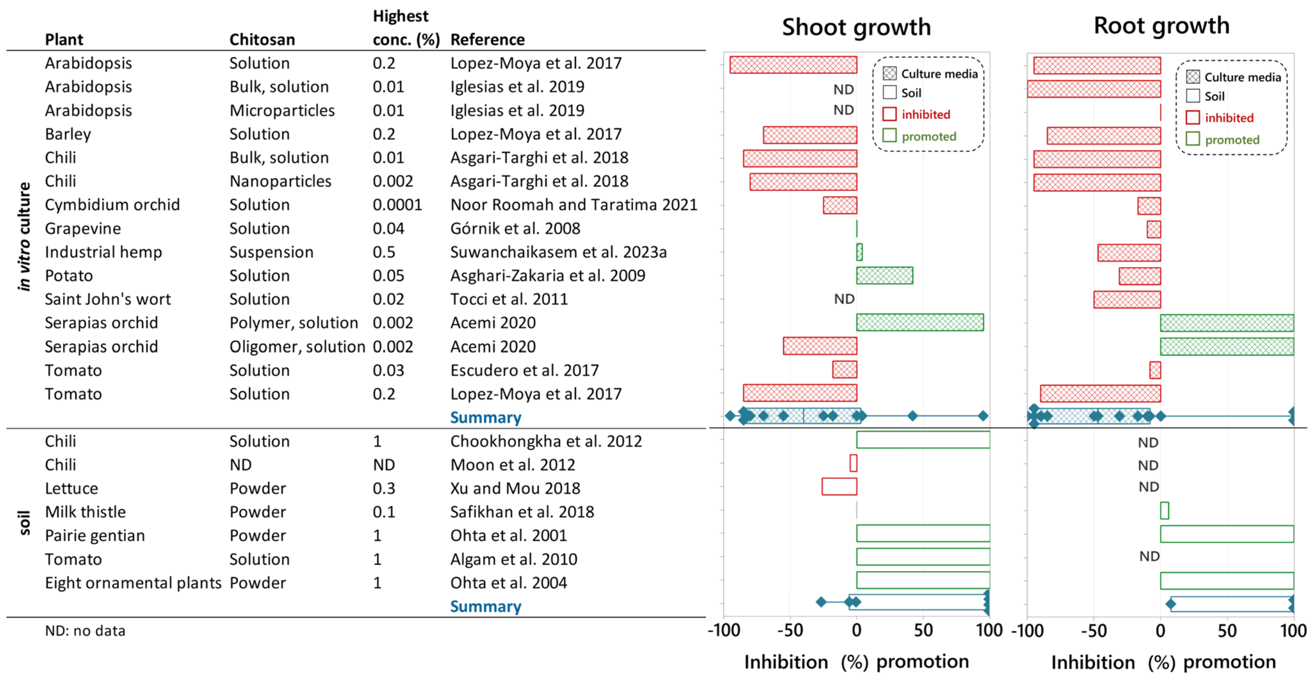

Fig. 2 Effects of chitosan on shoot and root growth among various plants and experimental settings. Based on experimental settings, all studies are classified into two groups; in vitro culture using growth media and potting system using soil. Bar graphs show approximate percentages of shoot and root growth inhibition (in red) or promotion (in green) upon the highest concentration of chitosan treatment within the particular study. Box plots (in teal), comprising interquartile range box and whiskers, show summary of each group. "ND" refers to not determined

weight were significantly increased in the low concentrations of chitosan treatment (0.05–0.2%) but all reduced in 0.3% chitosan treatment. The fresh weights were increased by 26–39% for 0.05–0.2% chitosan treatments but 26% decreased in 0.3% concentration (Xu and Mou 2018). In tomato (*S. lycopersicum*), after 30 days grown in sand irrigated daily with nutrient solution supplied with chitosan, shoot dry weights were increased by 31% in 0.005% chitosan treatment but reduced by 19% in 0.03% chitosan treatment. The reduction was more obvious in the condition containing beneficial nematode parasite, *Pochonia chlamydosporia*, where a 58% decrease of shoot dry weight was detected in the highest concentration (0.03%) of chitosan treatment (Escudero et al. 2017). In chili (*C. annuum*), after grown in soil drenched with chitosan, size and weight of individual fruit were not different from control but the number of ripen fruit and total fruit yield were significantly reduced by 5–7% (Moon et al. 2012). In tomato (*S. lycopersicum*) and barley (*Hordeum vulgare*), after 21 days grown in sand with daily irrigation of nutrient solution supplemented with chitosan, shoot fresh weights were reduced by approximately 30% in 0.05% chitosan and more than 50% in 0.1–0.2% chitosan conditions, but the reduction was not observed in the low concentrations of chitosan treatment (0.001–0.01%) (Lopez-Moya et al. 2017). In milk thistle (*Silybum marianum*), after 72 days grown in soil mixed with 0.01–0.1% chitosan, plant height, shoot dry weight, and total biomass were comparable to control. However, improvements were detected in chitosan treatments under salinity conditions, for example, shoot dry weight was increased by 20–40% in chitosan treatment under mild salt stress (4 dS m^{-1}) (Safikhan et al. 2018). In cucumber (*Cucumis sativus*), after 2–6 days of growth in hydroponic solution, shoot developments of the chitosan-treated plants (0.01–0.04% chitosan) were described as more vigorous than the untreated plants (El Ghaouth et al. 1994). In industrial hemp (*Cannabis sativa*), after eight days grown in hydroponic solution with 0.1–0.5% chitosan, total shoot fresh weight was not different to control (Suwanchaikasem et al. 2023a).

In tissue culture or in vitro experiments, the results of root chitosan treatment on shoot growth were also ambiguous. In *Arabidopsis thaliana*, after 21 days grown in the nutrient agar amended with chitosan, shoot fresh weights were approximately 40% lower than control in the lower doses of chitosan treatment (0.001–0.01%) and more than 80% decreased in the higher doses of the treatment (0.05–0.2%). The number of leaves per plant was also reduced by 30–50% in 0.05–0.2% chitosan treatments (Lopez-Moya et al. 2017). In protocorm culture of aloe-leafed cymbidium orchid (*Cymbidium aloifolium*), after 10 weeks cultured in the solid media supplied with chitosan, shoot height and total fresh weight were not different from control in the lower doses (0.05–0.1 ppm) but decreased by approximately 40% in the highest dose (1 ppm) of chitosan treatment. Number of leaves per protocorm was promoted in the lowest dose (0.05 ppm) but reduced in the highest doses (1 ppm) by approximately 25% (Noor Rohmah and Taratima 2021). In protocorm culture of long-lipped tongue orchid (*Serapias vomeracea*), the protocorm was grown in solid media supplemented with two types of chitosan, long-chain polymer (containing 70 subunits) and short-chain oligomer (with 2–15 subunits). The measurement was carried out 180 days after incubation. Among four doses of long-chain chitosan treatments, shoot length was significantly increased by approximately 1.8–2 times in the highest doses of 15–20 ppm concentrations but significantly decreased in the lowest dose of 5 ppm. In contrast, shoot length was increased in the lowest dose (5 ppm) of chitosan oligomer but significantly decreased in the higher doses of 15–20-ppm oligomer (Acemi 2020). In grapevine (*Vitis vinifera*), dipping cuttings into 0.01–0.02% chitosan solution for 24 h before planting improved number of internodes and new canes by approximately 10–30% and length of canes by approximately 30–40%, but the increments were not observed in the highest concentration (0.04%) of chitosan treatment (Górnik et al. 2008). In chili (*C. annuum*) grown in nutrient media supplemented with crude chitosan and chitosan nanoparticles, low concentrations (10–20 ppm) of crude chitosan improved total leaf area, leaf biomass, and shoot dry weight by 15–60%, but the highest concentration (100 ppm) inhibited shoot development by approximately 90% in all growth parameters. The similar outcome was also observed from chitosan nanoparticles, where all shoot growth parameters were significantly increased in the lowest dose of 1 ppm but 70–90% decreased in the higher doses of 5–20-ppm chitosan treatments (Asgari-Targhi et al. 2018). In potato (*Solanum tuberosum*) plantlets, after 21 days grown in solid media supplemented with chitosan, shoot fresh weight was significantly increased by approximately 40% in the highest concentration (500 ppm) of chitosan treatment. However, the lower doses of chitosan concentrations (5–150 ppm) did not affect shoot biomass (Asghari-Zakaria et al. 2009).

Based on current evidence, it could be summarized that unlike shoot treatment, root treatment with chitosan does not always promote shoot growth. The effect is likely to rely on several factors, including chitosan concentration, timing, duration and frequency of application, plant species, and growth conditions. Treating roots with relatively low to moderate chitosan concentrations tends to promote shoot growth, but once the concentration goes beyond certain limits, root chitosan treatment is likely to have no effect or, in some cases, contribute a negative impact on shoot growth. The threshold of chitosan concentration in soil-based systems seems to be higher than that in hydroponic system or micropropagation. Chitosan treatment on root tissues is likely to show greater benefits toward shoot growth when plants are under stress conditions. However, more research is required for appraisal before drawing definite conclusions for the effects of root chitosan treatment on shoot growth.

Root Development is Inhibited by Chitosan Treatment

Several studies have consistently demonstrated that chitosan has a negative impact on root development (Lopez-Moya et al. 2019). This inhibitory effect was not apparent in soil or field experiments. However, it was clearly recognized from the studies using hydroponic or nutrient-based systems (Fig. 2 and Table 1). This could be because in soil, roots are hidden underground and difficult to monitor, whereas in nutrient-based settings, roots can be readily observed through transparent liquid or solid media.

After 21-day cultivation in solid media, root growth of Arabidopsis seedlings was interrupted by the higher doses (0.01–0.2%) of chitosan treatment. The inhibitory effect was likely to be dose dependent since low doses of chitosan (0.001–0.005%) did not affect root growth, but 0.01% chitosan treatment reduced total root length by approximately 15%. The effect was strongest in the highest doses of 0.1% and 0.2% chitosan, where total root lengths of those conditions were more than 80% shorter than control (Lopez-Moya et al. 2017). A similar outcome was observed in tomato (*S. lycopersicum*) and barley (*H. vulgare*), where the low doses of 0.005–0.01% chitosan slightly arrested root growth by approximately 25%, but the higher doses of 0.1–0.2% chitosan showed more than 50% and 70% reductions of total root length and root fresh weights, respectively (Lopez-Moya et al. 2017). Another Arabidopsis study showed a similar result, where primary root lengths of Arabidopsis seedlings grown in solid media supplemented with the lower doses of 0.1–1 ppm chitosan were comparable to control. However, the plants grown in the media supplemented with the higher doses of 10–100-ppm chitosan showed more than 70% shorter primary root length than control after 3 days of treatment. The lateral root length and root number of the highest dose of 100-ppm chitosan were also significantly lower than those of control (Iglesias et al. 2019). In industrial hemp (*C. sativa*) grown in hydroponic solution, root growth was inhibited by 0.1–0.5% colloidal chitosan treatments. After eight days of treatment, total root length and surface area of chitosan-treated plants were more than 50% shorter and smaller than control (Suwanchaikasem et al. 2023a). In cymbidium orchid (*C. aloifolium*), the protocorms cultivated in the media supplied with the low doses (0.05 and 0.1 ppm) of chitosan for 10 weeks showed comparable total root number and root length to control, whereas those parameters were significantly decreased by

Table 1 Effects of root chitosan treatment on root growth and defense responses across different plants and settings

Plant	Chitosan treatment	Growing platform	Root growth	Root defense	References
In vitro culture					
American ginseng (*Panax quinquefolius*)	50 µl of 5000-ppm chitosan solution to callus clump	Petri dish with MS solid media	No data	Increases in phenolic contents and defense enzyme activity	Rahman and Punja (2005)
Arabidopsis thaliana	10–2000-ppm chitosan solution supplied in media	Petri dish with MS solid media	Inhibited by high concentrations of 100–2000 ppm	Accumulation of defense hormones, SA and JA and upregulation of genes involving in SA and JA biosynthesis pathways	Lopez-Moya et al. (2017)
A. thaliana	0.1–100-ppm bulk chitosan and chitosan microparticle solution supplied in media	Petri dish with MS solid media	Promoted by 1–10 ppm chitosan microparticles, but inhibited by 100-ppm bulk chitosan	No data	Iglesias et al. (2019)
Barley (*Hordeum vulgare*)	10–2000-ppm chitosan solution supplied in media	Petri dish with MS solid media	Inhibited by high concentrations of 100–2000 ppm	No data	Lopez-Moya et al. (2017)
Chili (*Capsicum annuum*)	1–100-ppm bulk chitosan and 1–20-ppm chitosan nanoparticle solution supplied in media	Tissue culture in bottle with MS media	Promoted by 1 ppm chitosan nanoparticles, but inhibited by 100-ppm bulk chitosan and 5–20-ppm chitosan nanoparticles	Decrease in defense enzyme activities in 1-ppm chitosan nanoparticles and 20-ppm bulk chitosan	Asgari-Targhi et al. (2018)
Grapevine (*Vitis vinifera*)	Pre-treated with 100–400-ppm chitosan solution diluted in water	Peat and sand mix	Promoted by low concentrations of 100–200 ppm but unchanged by high concentration of 400 ppm	No data	Górnik et al. (2008)
Industrial hemp (*Cannabis sativa*)	1000–5000-ppm chitosan suspension supplied in nutrient solution	Root-TRAPR device with hydroponic solution	Inhibited by 1000–5000 ppm concentrations	Increase in defense hormone and metabolite levels, enzymatic activities, and secretion of defense proteins	Suwanchaikasem et al. (2023a)
Orchid (*Cymbidium aloifolium*)	0.05–1-ppm chitosan solution supplied in media	Protocorm culture with ND solid media	Inhibited by high concentration of 1 ppm chitosan	No data	Noor Rohmah and Taratima (2021)
Potato (*Solanum tuberosum*)	5–500-ppm chitosan solution supplied in media	Bottle with MS solid media	Promoted by low concentrations of 5–15 ppm, but inhibited by high concentrations of 50–500 ppm	No data	Asghari-Zakaria et al. (2009)
Prairie gentian (*Eustoma grandiflorum*)	10–200-ppm chitosan solution supplied in media	Bottle with MS solid media	Promoted as compared to lactic acid, the diluent, but not different from control	No data	Ohta et al. (2000)
Rice (*Oryza sativa*)	150-ppm bulk chitosan and chitosan nanoparticle solution supplied in media	Cell suspension culture in Erlenmeyer flask with Chu's N6 liquid media	No data	Increase in phenolic and flavonoid levels and antioxidant enzyme activities	Arya et al. (2022)
St. John's wort (*Hypericum perforatum*)	200-ppm chitosan solution supplied in media	Cell suspension culture in Erlenmeyer flask with MS liquid media	Decrease in root biomass	Increase in production and secretion of xanthone, an antioxidant compound	Tocci et al. (2011)

Table 1 (continued)

Plant	Chitosan treatment	Growing platform	Root growth	Root defense	References
Tomato (*Solanum lycopersicum*)	10–2000-ppm chitosan solution supplied in media	Petri dish with MS solid media	Inhibited by high concentrations of 50–2000 ppm	No data	Lopez-Moya et al. (2017)
Tomato (*S. lycopersicum*)	100–2000-ppm chitosan solution supplied in nutrient solution	Magenta box with Gamborg B5 solution	No data	Increase in secretion of defense hormones and metabolites into exudate	Suarez-Fernandez et al. (2020)
Tomato (*S. lycopersicum*)	Daily irrigating with 10–300-ppm chitosan solution	Micropot with sand and Gamborg B5 supplement	Promoted by medium concentrations of 50–100 ppm, but inhibited by high concentration of 300 ppm	No data	Escudero et al. (2017)
Woad (*Isatis tinctoria*)	50–400-ppm chitosan solution supplied in media	Hairy root culture in Erlenmeyer flask with MS media	No data	Increase in flavonoid productions	Jiao et al. (2018)
Soil pot system					
Date palm (*Phoenix dactylifera*)	Injecting roots with 10 µl of 100–1000 ppm chitosan solution	Pot with peat and sand mix	No data	Increases in phenolic contents and defense enzyme activities	El Hassni et al. (2004)
Milk thistle (*Silybum marianum*)	0.01–0.1% w/w chitosan powder mixed in soil	Pot with soil	Promoted by chitosan treatment	No data	Safikhan et al. (2018)
Prairie gentian (*Eustoma grandiflorum*)	1% w/w chitosan powder mixed in soil	Pot with soil and fertilizer	Promoted by chitosan treatment	Increased chitinase activity	Ohta et al. (2001); Ohta et al. (2000); Ohta et al. (1999)
Eight ornamental plants	1% w/w chitosan powder mixed in soil	Pot with soil	Promoted by chitosan treatment	No data	Ohta et al. (2004)

JA jasmonic acid, *MS* Murashige & Skoog, *ND* New Dogashima, *SA* salicylic acid approximately 20–50% in the highest dose (1 ppm) of chitosan treatment (Noor Rohmah and Taratima 2021). In St. John's wort (*Hypericum perforatum*), plants cultivated in liquid media supplemented with 0.02% chitosan showed significantly lower total root biomass than control since day three after treatment. At 15 days after treatment, total root biomass was decreased by approximately 50% (Tocci et al. 2011).

In some studies, root growth was promoted upon the treatments with low concentrations of chitosan but inhibited upon high doses of chitosan treatment (Fig. 2). In chili (*C. annuum*), seedlings were grown in solid media supplemented with bulk chitosan and chitosan nanoparticles. Total root fresh weight was increased by approximately 40–70% in the conditions of low chitosan concentrations, including 10–20-ppm bulk chitosan and 1-ppm chitosan nanoparticles. However, root growth was strongly inhibited by high chitosan concentrations of 100-ppm bulk chitosan and 5–20-ppm chitosan nanoparticles. Total root length and fresh weight were approximately 80–90% lower than control (Asgari-Targhi et al. 2018). In grapevine (*V. vinifera*), cane cuttings were dipped in chitosan solutions for 24 h before cultivated in sand mix. After 75 days of cultivation, the number of primary roots originating from the cuttings was approximately 20% more than that of the control in the low concentrations of 0.01% and 0.02% chitosan solutions. Nonetheless, the number of primary roots was not different from control in the highest dose of 0.04% chitosan solution (Górnik et al. 2008). In protocorm culture of serapias orchid (*S. vomeracea*) treated with chitosan long-chain polymer and short-chain oligomer, root length was significantly enhanced by both types of chitosan. In chitosan long-chain polymer, the maximum increase of total root length was observed in the highest dose (20 ppm) of chitosan treatment. However, in the presence of chitosan oligomer, the maximum increase was observed in the lowest dose (5 ppm) of chitosan with approximately a four-time increase. The highest concentration of chitosan oligomer (20 ppm) showed approximately 2.5 times increase of root growth (Acemi 2020). In potato (*S. tuberosum*) plantlet, root fresh weight was significantly increased by approximately 13–43% in the lowest doses (5–15 ppm) of chitosan treatment but significantly decreased by approximately 18–32% in the highest doses of 50–500-ppm chitosan after 21 days grown in solid media (Asghari-Zakaria et al. 2009).

In soil-based experiments, the impact of chitosan treatment toward root growth is variable (Fig. 2). In cucumber (*C. sativus*), secondary root length of the plants grown in soil amended with 0.04% chitosan was described as shorter but thicker than control after two weeks of treatment (El Ghaouth et al. 1994). In tomato (*S. lycopersicum*), plantlets were grown in sand with and without beneficial fungus, *P. chlamydosporia*, and irrigated daily with the nutrient solution supplemented with chitosan. After 30 days of treatment, the root fresh weight and maximum root length were increased by approximately 30–154% in the low doses of 50 and 100 ppm of chitosan concentrations. However, the highest dose of chitosan treatment (300 ppm) significantly reduced root fresh weight by 53% in the condition containing the fungus and by 9% in the condition without the fungus (Escudero et al. 2017). In milk thistle (*S. marianum*), root dry weight was increased by approximately 6–11% in the soils supplemented with 0.01–0.1% chitosan after 72 days grown in pot (Safikhan et al. 2018). In prairie gentian (*E. grandiflorum*), root dry weight of the plant grown in soil amended with 1% chitosan was increased by 3.9 times as compared to the plants grown in untreated soil after 10 weeks (Ohta et al. 2001). The increases of root growth were also observed in other ornamental plants upon 1% chitosan treatment, such as 3–4 times increased in total root length of Rieger begonia (*Begonia hiemalis*) and Italian bellflower (*Campanula fragilis*) as compared to the control plants grown in soil added with normal fertilizer (Ohta et al. 2004).

To summarize these observations, chitosan treatment is prone to inhibit root growth (Fig. 2 and Table 1). The inhibitory effect tends to be dose dependent as the higher doses of chitosan have shown a stronger effect than the treatment with the lower doses. In terms of the molecular mechanism explaining these observations, the expressions of genes related to root elongation, including *WOX5* and *AQC1*, were suppressed upon chitosan treatment in Arabidopsis seedlings. Whereas, genes involved in the auxin biosynthesis pathway, including *YUC2*, *AAO1,* and *AMI1*, were upregulated, but *PIN1*, an auxin efflux-related gene, was downregulated, resulting in an accumulation of auxins at the root meristem, causing discontinuation of root elongation (Lopez-Moya et al. 2017, 2019). This could be one of the reasons for root growth arrest following chitosan treatment. Apart from auxin accumulation, further exploration is required to identify other factors that could contribute to this phenomenon. Investigation of the molecular interplay between the root surface membrane and chitosan binding would provide essential information on the critical steps involved in plant recognition of chitosan. In addition, expanding the research of chitosan effects toward root growth in agricultural fields would contribute significant impact for chitosan application. The knowledge gained would be an essential asset for further study to characterize downstream signaling molecules and cascades, contributing to root growth inhibition caused by chitosan.

Root Defense is Induced by Chitosan Treatment

Many reviews have addressed the research on the potential of chitosan to induce plant defense (Katiyar et al. 2015; Malerba and Cerana 2016; Pichyangkura and Chadchawan

2015; Riseh et al. 2022; Sharif et al. 2018; Stasińska-Jakubas and Hawrylak-Nowak 2022; Xing et al. 2014). The reviews show that any method of chitosan application, including foliar spraying, pregerminated seed priming, post-harvest coating, soil amendment, and regular irrigation, all effectively promote plant defense. The reviews also collectively illustrate that different types of chitosan, i.e., low, medium or high molecular weights, short or long chains, normal-sized or nano-sized particles, and soluble or powder forms, all are capable of eliciting plant defense (Faqir et al. 2021; Sravani et al. 2023). The eliciting effect of chitosan has also been widely detected across different crops, grown in different settings, with plant responses analyzed using an array of different techniques (Malerba and Cerana 2018; Pichyangkura and Chadchawan 2015).

In terms of molecular mechanisms, the first step of chitosan triggering plant defense is likely the binding of chitosan with plant cell membrane receptors (Fig. 3). To date, the receptors that can bind specifically with a chitosan molecule have not been identified (Cord-Landwehr and Moerschbacher 2021). The only plant surface membrane protein found to interact with chitosan is a chitin elicitor receptor kinase (CERK), a receptor-like kinase that is activated by chitin oligosaccharides (Gubaeva et al. 2018; Yin et al. 2016). The receptor binding either by chitosan or chitin oligosaccharides, in association with activation of calcium influx via adjacent calcium channels, triggers downstream signaling processes, in which the levels of secondary messengers, i.e., nitric oxide (NO), hydrogen peroxide (H_2O_2) and cytosolic calcium ion (Ca^{2+}), and defense hormones including salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) are increased in plant cells (Chandra et al. 2017; Gong et al. 2020; Katiyar et al. 2015; Lin et al. 2005; Lopez-Moya et al. 2017). Intermediate proteins and transcription factors, such as proteins in WRKY and AP2/ERF families, are involved in the signaling processes of chitosan induction (Coqueiro et al. 2015; Povero et al. 2011; Sripinyowanich et al. 2023). Finally, the signal activates the expressions of defense genes in the nucleus, leading to increased production of defense proteins, enzymes, and metabolites (De Bona et al. 2021; Katiyar et al. 2015). Defense proteins and enzymes upregulated upon chitosan treatment are, for example, pathogenesis-related (PR) proteins, catalase, chitinase, peroxidase, polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and superoxide dismutase (SOD). Activated defense metabolites are, for instance, phytoalexins, flavonoids, and phenolic compounds (Katiyar et al. 2015; Pichyangkura and Chadchawan 2015; Sravani et al. 2023). Chitosan was a stronger elicitor than chitin to induce production and secretion of plant defense hormones and proteins in root tissues of industrial hemp (*C. sativa*) (Suwanchaikasem et al. 2023a). Chitosan treatment can also manipulate plant physical barriers by increasing callose deposition and lignification of the plant cell wall (Sravani et al. 2023). Consequentially, chitosan-induced defense mechanisms promote plant resistance against both biotic and abiotic stresses (Hidangmayum et al. 2019; Mukarram et al. 2023).

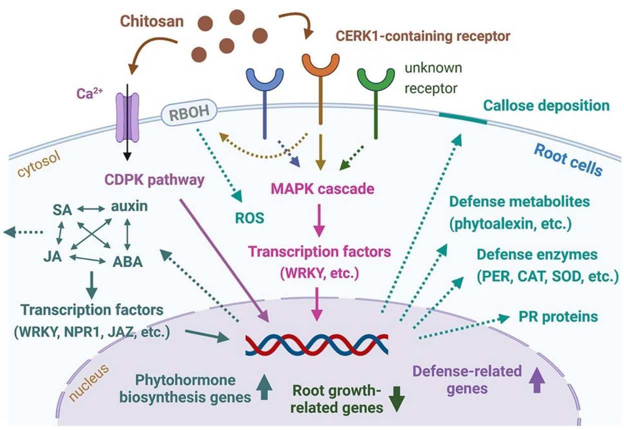

Fig. 3 Overview of possible molecular mechanisms underlying chitosan-manipulated plant defense and growth responses as modified from Das et al. (2015), García et al. (2021), Wang et al. (2020). Chitosan potentially binds to plant receptors, such as CERK1-containing receptor, to activate MAPK cascade and transcription factors to induce gene regulation in nucleus. Additionally, the binding of chitosan to the receptor is likely to increase reactive oxygen species (ROS) production via the activation of plant respiratory burst oxidase homolog (RBOH). Chitosan may promote calcium influx and activate the CDPK pathway to mediate gene regulation. Upon alteration of gene regulation, phytohormone biosynthesis may be affected, resulting in changes to cellular hormone levels and hormonal pathway crosstalk. Phytohormones, for example, auxins, salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) have intracellular roles that activate self-responses and intercellular functions signaling neighboring cells. Other phytohormones, such as brassinosteroids and gibberellins, may also play a part in the process. In the end, the defense-related genes may be preferentially upregulated, resulting in promotion of plant defense responses at the expense of plant root growth. Plants may strengthen their cell wall with callose deposition. Plants may increase production of defense metabolites and proteins, such as phytoalexin, peroxidase (PER), catalase (CAT), superoxide dismutase (SOD) and pathogenesis-related (PR) proteins in response to chitosan. *CERK1* chitin elicitor receptor kinase 1, *MAPK* mitogen-activated protein kinase, *CDPK* calcium-dependent protein kinase, *ROS* reactive oxygen species. This figure was created with Biorender.com

The research demonstrating plant defense-eliciting properties of chitosan specifically on root defense systems is summarized below and in Table 1. In Arabidopsis, the levels of phytohormones including SA, JA, and indole-3-acetic acid (IAA) were 2.5–3.6 times increased, and expressions of genes involved in SA and JA biosynthetic pathways, including *ICS1*, *ICS2*, *NPR1*, *AOC3*, *CYP94B*, and *MYC2*, were upregulated in root tissues treated with 0.1% chitosan within 24 h (Lopez-Moya et al. 2017). In industrial hemp (*C. sativa*), roots treated with 0.2–0.5% chitosan showed increases of total chitinase activity and defense hormone levels, including JA and its derivatives, JA-isoleucine (JA-Ile) and 12-oxo-phytodienoic acid (OPDA), by approximately 2–5 times. Chitosan treatment also induced secretions of defense proteins into the root exudate (Suwanchaikasem et al. 2023a). In tomato (*S. lycopersicum*), 0.1% chitosan treatment was demonstrated to impair root plasma membrane to induce secretions of defense hormones, including SA, JA and ABA, and growth hormone, IAA, into exudate. Secretions of lipid signaling molecules and defense-related metabolites were also increased after 20 days of chitosan treatment. The exudate with increased secretion of defense metabolites showed 1.5 times reduction of eggs hatched by root-knot nematode, *Meloidogyne javanica* (Suarez-Fernandez et al. 2020). In date palm (*Phoenix dactylifera*), roots injected with 0.01–0.1% chitosan solution showed increases in peroxidase and PPO enzymatic activities within 10–20 days after treatment. The levels of phenolic compounds including caffeoylshikimic acid, sinapic, ferulic, and *p*-coumaric derivatives in root tissues were also increased within 20 days (El Hassni et al. 2004). In ginseng (*Panax quinquefolius*), callus cultured in the media supplied with 1% chitosan showed 7 times increase of PPO activity and 1.5 times increase of total phenolic contents within 12 h after treatment (Rahman and Punja 2005). Based on microscopic observation, treating cherry tomato (*Lycopersicon esculentum*) roots with 0.1% chitosan in combination with an endophytic bacteria, *Bacillus pumilus*, induced callose depositions onto the inner cell wall surface, resulting in mitigation of *Fusarium oxysporum* fungal infection (Benhamou et al. 1998). In hairy root culture of woad (*Isatis tinctoria*), adding 0.015% chitosan into suspension media significantly increased total flavonoid production and antioxidant activity. The peak increment was at 36 h after the treatment, where total flavonoid content in the hairy root extract was increased by approximately five times. Individual contents of major flavonoids, such as rutin, quercetin, and isorhamnetin, were increased by approximately 7–13 times (Jiao et al. 2018). In St. John's wort (*H. perforatum*), root cultures elicited with 0.02% chitosan showed increases in xanthone production and secretion. The increases were highest at 7 days after treatment, where intracellular and extracellular xanthone levels were approximately 3.5 and 2.5 times higher than control, respectively. The increase of xanthone level in root tissues resulted in enhanced antifungal activity against fungal human pathogens, such as *Candida* spp. and *Cryptococcus neoformans* (Tocci et al. 2011). In rice (*Oryza sativa*) cell suspension cultures, supplying growth media with 0.015% bulk chitosan and chitosan nanoparticles significantly increased total phenolic and flavonoid contents and total activities of antioxidant enzymes, PAL, catalase, and peroxidase in the callus extracts at all time-points of 7, 14, and 28 days after treatment (Arya et al. 2022).

Rather than monitoring defense mechanisms, some studies demonstrated the effects of root chitosan treatment directly on plant resistance against pathogen infections. In tomato (*S. lycopersicum*) plantlets, daily irrigation with nutrient solution supplemented with 0.01% chitosan promoted root colonization of beneficial fungus, *P. chlamydosporia,* leading to reduction of disease severity caused by root-knot nematode, *M. javanica*. After 56 days of infection, plant shoot and root weights of the chitosan-treated plants were significantly higher than the infected plants without chitosan treatment (Escudero et al. 2017). In another study of tomato (*S. lycopersicum*), irrigating soil with 0.15% nano-chitosan five days before or during the infection reduced gall number of root-knot nematode, *Meloidogyne incognita*, on root tissues by 56–68%. After 47 days of infection, shoot and root fresh weight of chitosan-treated plants were approximately 23–29% higher than the infected plants without treatment and relatively comparable to the uninfected plants (Khalil et al. 2022). In carrot, mixing soil with 1% chitosan reduced disease incidence caused by pathogenic fungi: *Rhizoctonia solani* by 39.2% and *Athelia rolfsii* by 29.9%. Carrot yields of chitosan-treated plants were improved by approximately 70–90% upon *R. solani* and *A. rolfsii* infection as compared to the infected plants without chitosan treatment (Rahman et al. 2021).

All the evidence above confirms the capability of chitosan to induce root defense responses, leading to increased disease resistance against pathogens (Table 1). Chitosan also has additional benefits to disease control with its implicit antimicrobial effects against some species of bacteria, fungi, and viruses (Chandrasekaran et al. 2020; Raafat and Sahl 2009; Riseh et al. 2022). In addition, chitosan treatment can promote plant tolerance against abiotic stresses, such as salinity and drought (Arif et al. 2021; Hidangmayum et al. 2019). These additional properties add support for implementing chitosan in practical agriculture to manage plant biotic and abiotic stresses.

Growth-Defense Tradeoff is a Result of Root Chitosan Treatment

By integrating information from "Root Development is Inhibited by Chitosan Treatment" and "Root Defense is Induced by Chitosan Treatment" sections, it could be concluded that chitosan inhibits root growth but activates root defense (Table 1 and Fig. 3). Once plant roots are exposed to chitosan, root activity is likely to shift from habitual root expansion to consolidating defense systems. This process is termed the "growth-defense tradeoff," which is an important mechanism enabling plants to adapt and survive in uncertain environmental conditions (He et al. 2022). Both biotic and abiotic stresses are external factors able to turn on or off this alteration (Figueroa-Macias et al. 2021). For example, in the situation when a young seedling is searching for light but is under a dense canopy, it may heighten shoot growth quickly and minimize defense, thereby becoming increasingly vulnerable to pathogen attacks. On the other hand, a mature plant when attacked by a fungal pathogen may promptly activate a hypersensitive defense response and sacrifice the infected cells to limit the spread of the infection. In this case, plants may stop extending physical growth (Agrios 2005; He et al. 2022).

Molecular mechanisms underlying this tradeoff are not fully understood but are likely attributed to the interaction between growth and defense signaling pathways (Eichmann and Schafer 2015). Current understanding in the mechanisms of growth versus defense balance has been reviewed, with the key players proposed being phytohormones (Denancé et al. 2013; Dhar et al. 2020; Huot et al. 2014; Li et al. 2022; Lozano-Durán and Zipfel 2015). Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are defense hormones, actively functioning once plants encounter dangers, while auxin, cytokinin, gibberellin (GA), and brassinosteroid (BR) are mainly responsible for growth and development. Nonetheless, they crosstalk to each other and work in concert to manage stresses and maintain plant life (Eichmann and Schafer 2015; Huot et al. 2014; Lozano-Durán and Zipfel 2015). Endogenous phytohormones work like messengers, receiving primary signals to cell membrane receptors and circulating the messages intracellularly to activate gene expressions in nucleus and extracellularly to neighboring cells to communicate the coming of dangers (Berens et al. 2017; Denancé et al. 2013). Activation of mitogen-activated protein kinase (MAPK) cascades upon the binding of chitosan to CERK1 receptor is another important mediator, transducing signals from cell surface to trigger gene transcriptions and protein translations in nucleus (Zhang et al. 2018). Several transcription factors are involved in the processes of chitosan-induced plant growth and defense responses: for example, NPR1, a receptor and regulator of SA signaling; DELLA-family proteins, repressors of GA signaling; JAZ-family proteins, suppressors of JA signaling; and BIN2 kinase, a repressor of BR signaling (Eichmann and Schafer 2015; Huot et al. 2014). Additionally, some transcription factors, for example, homolog of brassinosteroid-enhanced expression2 interacting with IBH1 (HBI1), DP-E2F-like 1 (DEL1) and TL1-binding transcription factor 1 (TBF1), are identified as intermediate molecules, situating between growth and defense pathways and functioning to balance plant growth and defense activities (Chandran et al. 2014; Neuser et al. 2019; Pajerowska-Mukhtar et al. 2012; Wang and Wang 2014). Eventually, signaling processes end in the nucleus, where expression of growth- and defense-related genes are regulated accordingly. Circadian clock-associated 1 (*CCA1*) and *YUCCAs* genes are examples of growth-related genes, and pathogenesis-related proteins (*PRs*), ethylene response factor (*ERFs*), and plant defensins (*PDFs*) are some defense-related genes, activated upon growth-defense tradeoff (Cerrudo et al. 2017; Groszmann et al. 2015; He et al. 2022). Nonetheless, further study is required to convert this level of molecular understanding in plant growth-defense tradeoff to the natural conditions, where plants could be subjected to both biotic and abiotic stresses; to return to an earlier example, identifying what signaling pathways are affected when a plant is searching for light and meanwhile attacked by a pathogen (He et al. 2022).

As discussed in "Root Development is Inhibited by Chitosan Treatment" and "Root Defense is Induced by Chitosan Treatment" sections (Table 1), exogenous chitosan can drive this shift from growth to defense in plant roots by arresting root growth and activating defense responses by increasing production of defense metabolites and proteins and thickening cell wall barriers (Lopez-Moya et al. 2019; Sofi et al. 2023). However, plant growth and defense pathways affected by chitosan have not been completely identified. Chitosan may trigger typical plant defense signaling cascades. The process starts when danger signals from pathogens, called pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), bind to plant cell surface receptors, named pattern recognition receptors (PRRs). The binding activates downstream signaling pathways and eventually induces the production of defense-related compounds. This process is termed the PAMP-triggered immunity (PTI) (Ramirez-Prado et al. 2018; van der Burgh and Joosten 2019). Danger signals to activate PTI are, for example, bacterial flagellin, bacterial elongation factor-Tu (EF-Tu) or fungal chitin (Zhang and Zhou 2010). Chitosan has not been well recognized to bind with PRRs and induce the PTI (Yin et al. 2016). CERK1 is the only plant cell membrane protein identified to date to be associated with the response to chitosan exposure (Gubaeva et al. 2018; Sofi et al. 2023). In root tissues, CERK1 was only known to be regulated upon fungal attack and salt stress (Espinoza et al. 2017; Zhang et al. 2015). Further exploration is required to identify whether enhanced defense responses in root tissues according to chitosan treatment is activated via the interaction with root CERK1 receptor.

In Arabidopsis, root chitosan treatment affected the expression of genes involved in biosynthesis of defense hormones, SA and JA, including *ICS1*, *ICS2*, *NPR1*, *AOC3*, *CYP94B,* and *MYC2*, causing increased levels of SA and JA hormones in root tissues. In industrial hemp (*C. sativa*), the levels of root ABA, JA, JA-Ile, and OPDA, were increased upon chitosan treatment (Suwanchaikasem et al. 2023a, b). Defense hormones, including SA, JA, and ABA were increasingly secreted into root exudate of tomato (*S. lycopersicum*) treated with 0.1% chitosan (Suarez-Fernandez et al. 2020). These findings signify that chitosan treatment manipulates defense hormone levels in root tissues. On the other side, chitosan can regulate auxin biosynthesis pathways. In Arabidopsis, genes involved in auxin biosynthesis, including *YUC2*, *AMO1,* and *AMI1*, were upregulated, but genes related to auxin efflux, *PIN1*, and root elongation, including *WOX5* and *AQC1*, were downregulated upon chitosan treatment, causing auxin accumulation at the root tip (Lopez-Moya et al. 2017). This result suggests that the arrest of root growth in response to chitosan could be activated via auxin hormone signaling pathways.

After all, further research is required to elucidate and define in detail the molecular pathways triggered by chitosan and find the connection between growth and defense hormone crosstalk to fill in the gaps left in Fig. 3. A comprehensive understanding of chitosan effects toward plant root systems would provide a solid evidence basis for the application of chitosan in agriculture. It would also benefit the crop improvement sector who could use this knowledge to identify plant traits that produce high yield and still maintain high disease-resistance profiles (Cunha da Silva et al. 2019).

Methods to Apply Chitosan Onto Plant Root Systems

To date, the effects of chitosan on plant roots have been mostly examined in controlled laboratory settings. Two types of setups were used: soil based and nutrient based. In soil-based experiments, chitosan powder was usually directly mixed with the soil by weight in a concentration of 0.01–1% w/w (Ohta et al. 2004; Safikhan et al. 2018; Xu and Mou 2018). In nutrient-based experiments, chitosan was generally dissolved in weak acids, such as acetic, formic, or lactic acids, and then mixed with plant growth media, such as Murashige and Skoog (MS) medium (Asgari-Targhi et al. 2018; Jiao et al. 2018). In some cases, chitosan was prepared in a colloidal form by dissolving in a strong acid, such as hydrochloric acid (HCl) or orthophosphoric acid (H_3PO_4), to create a colloidal chitosan suspension. The acidic condition is then neutralized using basic compounds and/or vigorously washed with water to remove excess salts and acids. It could then be dehydrated and finally re-suspended in water, nutrient solution, or low-concentration weak acids (Olicón-Hernández et al. 2017; Palma-Guerrero et al. 2008; Suwanchaikasem et al. 2022). In a few cases, chitosan hydrochloride is created to enhance solubility of chitosan in water (De Vega et al. 2021). The methods of chitosan application on plant root systems are summarized in Fig. 4.

Ideally, chitosan would be dissolved in an aqueous-based solution, which would then enable easy addition to soil components or plant growth media to achieve root treatment. However, chitosan is a water-insoluble polymer, causing some barriers to the application. Chitosan is, in turn, soluble in acid due to its free amino group in the D-glucosamine subunit, which can be readily protonated under acidic conditions, with $pK_a \approx 6.3$ (Aranaz et al. 2021; Jimenez-Gomez and Cecilia 2020). The solubility of chitosan varies on its chemical properties, such as size, molecular weight, and degree of deacetylation. For example, the higher degree of deacetylation or the higher number of free amino acid groups in chitosan polymeric structure, the easier dissipation

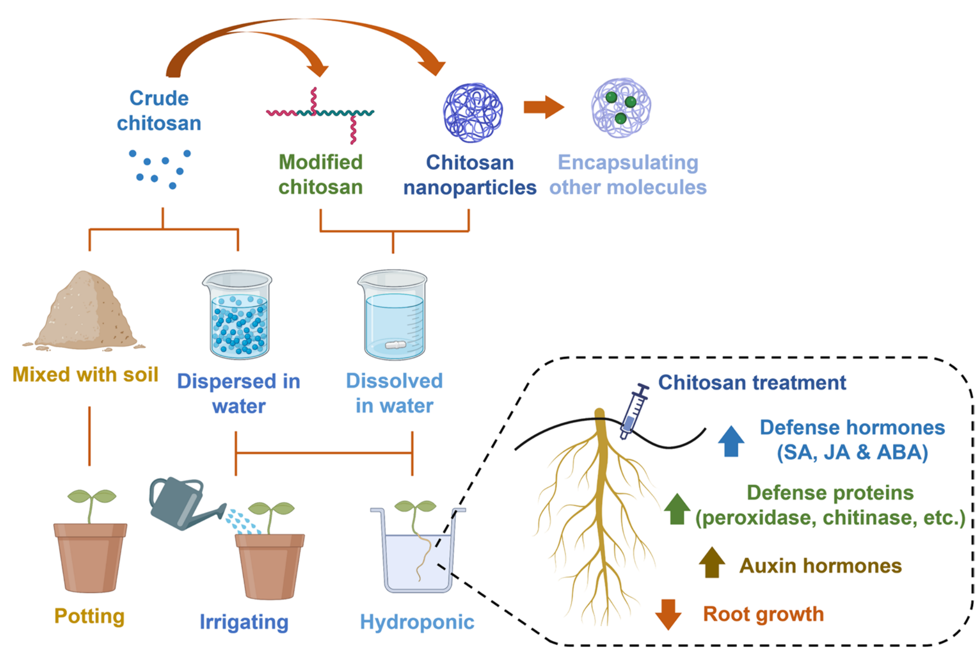

Fig. 4 Different techniques of chitosan application on plant root systems and the summary of plant growth and defense responses upon root chitosan treatment. This figure was created with the assistance of Biorender.com

of chitosan polymer (Dash et al. 2011). Nonetheless, solubilizing chitosan in acid solutions creates a mild acidic condition, pH 4–5, which can affect plant development. For example, supplying 0.5% acetic acid in MS medium reduced chili (*C. annuum*) height and number of leaves by 46% and 35%, respectively. When plant roots were treated with 20-ppm low-, medium-, and high-molecular weight chitosan, dissolved in 0.5% acetic acid, root growth was significantly interrupted, which could be a result of media acidity (Chookhongkha et al. 2012). In prairie gentian (*E. grandiflorum*) culture, supplying MS medium with 0.0025% lactic acid decreased root dry weight by 50%. In the root treatment with 0.001–0.02% chitosan, dissolved in 0.0025% lactic acid, root dry weight was significantly higher than the acid media control but not different from the neutral media control (Ohta et al. 2000). Soil acidity is also known to restrict plant growth because of decreased availabilities of essential nutrients, such as phosphorus, calcium, and molybdenum, and increased levels of certain ions, such as aluminum and manganese to toxic levels (Msimbira and Smith 2020). Soil acidity also affects plant–microbe dynamics, which could alter symbiotic activity between plant roots and surrounding organisms. Therefore, the profits that plants would gain from beneficial microbes, such as nitrogen-fixing bacteria, would be mitigated (Msimbira and Smith 2020). Moreover, dissolving natural chitosan, prepared from black soldier fly (*Hermetia illucens*), in 1% acetic acid changed the polymeric structure of chitosan, reducing beneficial properties of chitosan in the context of thermal stability, crystallinity, and hydrophobicity (Bilican et al. 2020).

To avoid using acids for chitosan dissolution, several techniques have been applied to improve chitosan solubility, which is important for chitosan application onto plant root systems, especially with irrigation and supplementation into hydroponic solution (Fig. 4). Ionic liquids are an alternative solvent for dissolving chitosan (Li et al. 2019; Zhang et al. 2021). The technique uses non-volatile and reusable solvents, e.g., 1-ethyl-3-methylimidazolium acetate and 1-butyl-3-methylimidazolium chloride, which are considered safe and non-toxic to humans and the environment (Inamuddin and Asiri 2020). However, the method requires further optimization to make it cost-effective for mass production and, more importantly, critical evaluation on the side effects toward plant growth and surrounding environments (de Jesus and Maciel Filho 2022; Goncalves et al. 2021).

Chitosan powder can be transformed into chitosan micro/nanoparticles to improve chitosan physical and chemical properties (Colman et al. 2019; Iglesias et al. 2019). The transformation requires additional steps of preparation but obtains an improved version of chitosan with increased consistency, surface area, and solubility (Mohammed et al. 2017). It also improves chitosan efficiency, in which chitosan nanoparticles can be applied at a lower concentration within a range of 0.001–0.01% w/v, while the applied range of normal-sized chitosan is around 0.01–1% w/v (Asgari-Targhi et al. 2018). Chitosan micro/nanoparticles also have an advantage to incorporate other substances into their core structure, mimicking drug delivery systems to deliver active compounds to target sites. The incorporated compounds are protected from pH instability, enzyme degradation and other detrimental environmental conditions by chitosan nanostructure (Imam et al. 2021; Saberi Riseh et al. 2022a). After transformation, chitosan micro/nanoparticles do not lose plant defense-eliciting and antimicrobial properties but, in turn, show better performance in pest and disease control (Kumaraswamy et al. 2018; Sravani et al. 2023).

Modifying chitosan polymeric structure is another way to improve solubility and biochemical properties (Khan and Alamry 2021; Silva et al. 2021; Zhao et al. 2018). Due to free hydroxyl and amino groups in the D-glucosamine subunit, the polymer can undergo various chemical reactions, such as hydroxylation, alkylation, acylation, esterification, and carboxylation. The modified versions of chitosan, for example, carboxymethyl chitosan and quaternary ammonium chitosan, have greater water-soluble properties than intact chitosan, broadening their applications in biomedical and pharmaceutical fields (Fabiano et al. 2020; Shariatinia 2018). Another technique is a polymer grafting to merge chitosan with other polymers, for example coupling chitosan with polyethylene glycol or oligo lactic acid (Khan and Alamry 2021; Zhao et al. 2018). These chitosan copolymers show an improvement in controlling drug release when used as drug carriers, benefiting research into drug delivery, enhancing physical and biological properties of drug vehicles (Bhattarai et al. 2006; Wang et al. 2008). The possibility of using these new chitosan complexes on plant roots, especially when used in combination with other compounds, is discussed in the next section. Nonetheless, the new chitosan formulations need intensive research to assess their advantages and drawbacks toward plant development before being introduced in practical application.

To summarize this section, water insolubility of chitosan raw material is the major issue of root chitosan treatment. Based on current utilizations, chitosan is either diluted in weak acids, formed salt with strong acid, dispersed in hydroponic solution or directly mixed with soil or organic substrates. To improve chitosan solubility and biological functions, chemical modifications, such as transforming chitosan into nanoparticles, modifying chitosan core structure, or integrating chitosan with other polymers have been studied. However, further research is required to thoroughly examine the effects of the new chitosan materials on plant growth and defense responses. In addition, they might not be currently available and affordable for local users. Therefore, developing simplified procedures for chitosan production and preparation would be essential to underpin the practical usage of chitosan in crop productions.

Combination of Chitosan Treatment with Other Methods for Fungal Disease Control

The combination of multiple treatments onto plant root systems has been demonstrated to enhance the efficiency of disease control (Ons et al. 2020). Several techniques can synergize biological effects. For instance, natural products can be combined with synthetic chemicals to promote plant defense in parallel with targeting the invading pathogens. For example, applying a beneficial fungus, *Trichoderma atroviride,* in addition to drenching potting substrates with 0.01% fluazinam fungicide enhanced the chance of disease control by approximately 10–30% as compared to the use of fungicide alone to manage white root rot in avocado (*Persea americana*) (Ruano-Rosa et al. 2018). Drenching soil with beneficial bacteria, *Burkholderia cepacia* or *Bacillus megaterium*, along with 0.01% carbendazim fungicide reduced disease occurrence on tomato (*S. lycopersicum*) by approximately 50–60% as compared to fungicide treatment alone (Omar et al. 2006). Combining different microbes to build microbial consortia has also been revealed to enhance disease control performances against soil-borne fungal pathogens (Xu et al. 2011). The fungal and bacterial species commonly used to form microbial consortia are, for instance, *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Azospirillum* spp. Combination of these microbes are effective to prevent infections from fungal pathogens, for example, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Rhizoctonia solani,* and *Athelia rolfsii* (Palmieri et al. 2016; Sarma et al. 2015).

Chitosan has also been applied in combination with other biocontrol agents such as beneficial bacteria, fungi, seaweed extract, essential oils, and phytocompounds to manage plant diseases. For example, drenching soil with 0.01% w/v chitosan and 0.5% or 1.5% v/v brown algae (*Ascophyllum nodosum*) extract significantly reduced disease incidence and area of infection of Fusarium head blight, caused by *Fusarium graminearum*, in wheat (*Triticum aestivum*) leaves by approximately 30–80% as compared to individual chitosan or brown algae extract treatment (Gunupuru et al. 2019). Amending soil with 0.1% chitosan alone reduced disease severities of Fusarium seedling blight, caused by *F. culmorum*, by approximately 30% in wheat (*T. aestivum*) and 70% in barley (*H. vulgare*). By adding beneficial bacteria, *Pseudomonas* sp. MKB 158 or *P. fluorescens* MKB 249, into soil, disease incidence was approximately 20–50% lower than the chitosan treatment alone (Khan et al. 2006). Pre-treating tomato seedlings (*S. lycopersicum*) with 0.05% or 0.1% w/v chitosan and beneficial fungus, *Trichoderma harzianum* diminished disease incidence caused by *F. oxysporum*, by 50% as compared to the untreated plants and approximately 16–40% as compared to the single treatment of chitosan or beneficial fungus (El-Mohamedy et al. 2014).

To facilitate the use of chitosan in combination with other biocontrol agents, chitosan can be transformed into nanoparticle formulations to incorporate other biocontrol agents into nanocore structure, creating synergy in disease control. This technique has been investigated in biomedicine using chitosan nanoparticles as a drug carrier to encapsulate and deliver active compounds to target sites (Patra et al. 2018; Sung and Kim 2020). Chitosan nanoparticles can be prepared using various techniques. The preparation procedures along with advantage and drawback of each method have been extensively reviewed in many articles (Divya and Jisha 2017; Kumaraswamy et al. 2018; Singh et al. 2021; Yanat and Schroën 2021). The most favorable method is an ionic gelation, where chitosan powder is dissolved in acetic acid and then cross-linked with tripolyphosphate (TPP). The active compound is gently added before or during the crosslinking step (Hoang et al. 2022). Alternatively, oil-in-water emulsification is a method for combining chitosan nanoparticles with hydrophobic molecules, such as essential oils, using Tween 80 as an emulsifying agent (Das et al. 2019; Hasheminejad et al. 2019; Hosseini et al. 2013). Apart from plant defense-eliciting property, chitosan also has bactericidal and fungicidal properties, giving an extra benefit to the composites (Ke et al. 2021; Xing et al. 2014). Additionally, chitosan can be integrated with metals, such as magnesium, iron, and copper, forming chitosan-metal nanocomposites to promote antimicrobial functions (Ahmed et al. 2021; Bharathi et al. 2019; Rubina et al. 2017; Wang et al. 2005).

Chitosan nanoparticles have been demonstrated to exceed the performances of normal-sized chitosan to promote plant defense (Saberi Riseh et al. 2022a,b). However, most of the studies tested the effects of chitosan nanoparticles on plant shoot tissues or in cell suspension cultures. For example, in rice (*O. sativa*) suspension culture, 0.015% chitosan nanoparticles significantly increased productions of phenolic and flavonoid compounds by approximately 2.5 times as compared to control and 1.5 times as compared to 0.015% crude chitosan after 14 days of treatment. The activity of antioxidant enzyme, phenylalanine ammonia lyase (PAL), was also increased 2.5 times in chitosan nanoparticle treatment as compared to control and 1.25 times as compared to crude chitosan. The levels of phenolics and flavonoids and PAL enzymatic activity were further increased in the treatment of chitosan nanoparticles loaded with methyl jasmonate (Me-JA), a defense hormone in JA pathway, as compared to unloaded chitosan nanoparticles and crude chitosan loaded with Me-JA (Arya et al. 2022). In soil experiment, soaking cherry tomato (*L. esculentum*) seeds in the solutions of chitosan nanoparticles merged with vanillin or cinnamaldehyde for 5 h prior to planting diminished disease severity index by 2.5–3.3 times and 1.7–2.8 times against *F. oxysporum* and *Pythium debaryanum* pathogens, respectively. After 60 days of infection, shoot fresh and dry weight and number of leaves of the treated plants were higher than those of the infected plants without treatment (Elsherbiny et al. 2022). Mixing chili (*C. annuum*) seeds with alginate-chitosan nanoparticle beads entrapping bacterium, *Bacillus licheniformis* decreased disease incidence by approximately 30% as compared to negative control (water) and empty alginate bead after three days of *A. rolfsii* infection on two-week-old seedlings. The study also showed that the alginate bead containing both chitosan nanoparticles and beneficial bacteria had better performance than the beads containing only chitosan or bacteria alone to promote chili shoot growth in the uninfected condition (Panichikkal et al. 2021). Spraying cherry tomato (*L. esculentum*) leaves with chitosan nanoparticles loaded with harpin, a natural protein elicitor extracted from *Pseudomonas syringae* bacteria, reduced disease lesions caused by fungal pathogen *R. solani* and the DNA copy number of the fungus on plant leaves by more than 70% as compared to the treatments with empty chitosan nanoparticles or harpin elicitor alone. The leaves sprayed with chitosan nanoparticles containing harpin protein also showed increased peroxidase and PAL activities as compared to the treatments with chitosan or harpin alone at 3–4 days after treatment (Nadendla et al. 2018). However, the effects of chitosan nanoparticles have been barely studied on plant root systems, requiring further research to fill this lacking information to verify the benefits of chitosan toward overall plant defenses.

Due to capability to incorporate other molecules into its polymeric structure, chitosan is in a great position for the use in combination with other biocontrol agents to promote plant defense and manage plant diseases. Additional steps of preparation and encapsulation are required to produce a chitosan composite integrated with other molecules. Therefore, further research is needed to streamline and standardize the procedures and to test the efficacy and potential adverse effects of encapsulated chitosan nanoparticles on plant root systems.

Conclusion and Perspective

One ultimate goal of studying chitosan in the context of plant defense activation and disease resistance promotion is to find a substitute for chemical pesticides and fungicides. Application onto plant root systems by mixing with soil, daily irrigation or adding to liquid nutrient, would be convenient methods for chitosan treatment. Considerable research has confirmed the eliciting effects of chitosan on plant root defenses. However, chitosan seems to have a drawback on root growth. Once exposed to chitosan, plant roots are likely to stop physical growth and turn on defensive pathways. This phenomenon has been observed from several studies, conducting plant growth experiments in an in vitro setup using transparent plant growth media as nutrients (see in "Root Development is Inhibited by Chitosan Treatment" section). Nonetheless, the result seems to be inconclusive in soil-based experiments, where several factors, such as growth conditions, timepoint and duration of chitosan application, and plant species could impact the effects of chitosan on both shoot and root growth. This requires further studies to verify and confirm the effects of chitosan on plants, especially agricultural crops, grown in the field under farmland conditions.

Chitosan can be prepared in different formats, such as chitosan suspension, chitosan nanoparticles, and grafted chitosan copolymers. A comparative study is needed to examine their positive and negative impacts on plant tissues side by side. The modified versions of chitosan, including chitosan nanoparticles and graft chitosan copolymers, show improvement in chitosan water-soluble properties, facilitating the application of chitosan on plant root systems. Nonetheless, further research is required to simplify the preparation pipeline to make the modified chitosan a cost-effective material. This would also be useful for large-scale chitosan production for other commercial purposes, such as developing chitosan nanoparticles as film coating or food packaging materials.

Before implementation of chitosan in pest and disease control in agriculture, research is required to confirm the efficacy of chitosan in comparison with currently available commercial fungicides and pesticides. At the earlier stage, chitosan could be used in combination with fungicides and pesticides to reduce the amount of chemicals. It could then be utilized in combination with other techniques, such as beneficial microbes, to promote plant defenses. Good farming practices, such as avoiding fields with disease history, rotating with non-host crops and regularly screening sites and immediately removing infected plants, are still fundamental methods to prevent disease occurrence. By combining these techniques with chitosan treatment, pests and diseases in crop cultivations could be controlled in a more sustainable way to reduce environmental impacts of fungicides and pesticides and improve well-being of farmers and consumers.

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Declarations

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