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Chitosan Enhances Germination and Growth of Rice Plant (*Oryza sativa*) while controlling *Rhizoctonia solani*
Infection

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Abstract

Rice is one of the world's major staple crops, and improving its germination and growth is paramount for food safety. In the present study, IR64 rice seeds were treated with commercial and biologically synthesized chitosan using *Streptomyces griseoincarnatus* to assess their impact on germination efficiency, growth performance, and disease resistance. Synthesized chitosan significantly reduced the mean germination time and T_{50} , but improved the germination index, suggesting quicker and more synchronous germination. Synthesized chitosan also encouraged more root and shoot growth, a higher number of tillers, wider leaves, biomass production, chlorophyll content, and ultimately better grain yield. Synthesized chitosan was highly active against *Rhizoctonia solani*, lowering the severity of disease and activating plant defense by elevating PR 10 gene expression by ~70-fold. The findings indicate that biologically synthesized chitosan acts both as a biostimulant and biocontrol agent and providing a biodegradable and renewable alternative to traditional agrochemicals for enhancing rice growth, productivity, and stress tolerance.

Keywords: Chitosan, IR64, *Rhizoctonia solani*, *Streptomyces griseoincarnatus*

1. Introduction

Rice (*Oryza sativa*) is a staple food in India and worldwide, serving as a primary food source for more than half of the global population. The production of rice was dwindling due to various natural calamities and microbial infections (Fahad et al., 2019). Ensuring vigorous growth and healthy establishment of rice seedlings is critical for achieving optimal yield and food security. Germination of seeds and early seedling development are highly vulnerable stages that affect the overall growth and development of the plant (Lamichhane et al., 2018). Also, less vigorous seeds are more stressed in acclimating to field environments. However, formulating a suitable pre-treatment with a bio-stimulant might laterally boost the plant's immune defence, and enhance resistance to pests and pathogens, resulting in higher yields.

In addition to this, the rice plant after growth faces significant biotic challenges, among which sheath blight disease caused by the fungal pathogen *Rhizoctonia solani* is one of the most devastating diseases, which results in more than 20- 50% of crop loss under severe outbreaks (Senapati et al., 2022). The traditional fungal control strategies heavily depend on chemical fungicides, which pose various health hazards, environmental pollution, and may contribute to the emergence of fungicide-resistant strains (Samal et al., 2024). Some of the chemical treatments can have a negative or inhibitory effect on the microbial flora and fauna, and this increases the effect of the fungal infection (Savvides et al., 2016). Henceforth, alternative compounds that can act as both a growth promoter and a fungicide need to be explored.

Chitosan, a deacetylated form of chitin primarily derived from the shells of crustacean animals and the cell walls of fungi, has gained significant attention in recent years due to its versatile characteristics and multifaceted applications in various fields. Its distinctive properties, which include biodegradability, biocompatibility, and non-toxicity, make it an ideal candidate for application in the protection of crops and growth promotion (Falcón-Rodríguez et al., 2012).

The present study aims to evaluate the effectiveness of chitosan derived from *Streptomyces griseoincarnatus* RB7AG along with commercial chitosan in promoting seed germination and inhibiting the growth of common

phytopathogenic fungi *Rhizoctonia solani*. Additionally, the experiment was designed to assess whether the synthesized chitosan shows comparable bioactivity to its commercial counterpart.

2. Materials and Methods

2.1. Growth and Maintenance of Organisms

Streptomyces griseoincarantus RB7AG was maintained in SWLB (Seawater Luria Bertani) media (Tryptone: 5g/L, Yeast extract: 2.4 g/L, NaCl: 2.4g/L, dH₂O: 500 mL, seawater: 500 mL). This medium was used as the pre-culture for chitosan production (Behera et al., 2023).

2.2. Preparation of colloidal chitin and chitosan production

Colloidal chitin was prepared according to the method described by Hsu and Lockwood 1975. Briefly, 240 mL of conc. HCl was added to 20 g of chitin flakes and left for 30 min. After 30 minutes of incubation, cold distilled water was added to the acidified slurry and sieved through filter paper to obtain the chitin precipitate, which was washed repeatedly until the pH reached neutral. The obtained precipitate was dried at 50-60°C, and distilled water was added to make a 2% colloidal chitin solution.

Mineral Salt Medium (MSM) (K₂HPO₄: 0.7g/L, KH₂PO₄: 0.35g/L, MgSO₄: 0.5g/L, MnCl₂: 0.01g/L, FeSO₄:0.001g/L, ZnSO₄:0.001g/L, NaCl: 0.5g/L) with 1% of the colloidal chitin and 0.8×10⁶ CFU/mL of *Streptomyces griseoincarantus* RB7AG was inoculated and incubated at 37°C for the production of chitosan. After 48 hours., the culture was centrifuged, and the supernatant was collected; chitosan was recovered by ammonium sulphate precipitation, washed three times with double-distilled water, and lyophilised to obtain the chitosan powder (Behera et al., 2024).

2.3. Seed collection and sterilization

Rice variety, IR64, was procured from the National Rice Research Institute (NRRI), Cuttack. The seeds were healthy and of the same dimensions. The seeds were surface-sterilized before use.

The rice seeds (100-200) were washed three times with autoclaved distilled water and soaked in 70% ethanol for 2-3 mins. After ethanol, it was immersed in 2% sodium hypochlorite and kept for 10-15 mins in a rocker. The seeds were washed thoroughly with distilled water to completely remove traces of sodium hypochlorite. Then the seeds were used for the experiment.

2.4. Seed priming and chitosan biostimulant effect

For the germination assay, 30 seeds for each treatment group (three replicates), i.e., Control (Only water), T1- Synthesized chitosan (1mg/mL), and T2- Commercial chitosan (1mg/mL), were soaked in 25 mL of the respective solution for 24 hours under dark conditions.

2 layers of germination paper were placed on a glass Petri dish. Then the overnight-soaked seeds were transferred to the plates (10 seeds/plate×3 replicates) of different groups. 5 mL of distilled water was added to the plate to keep it moist, and sealed with parafilm containing small holes for aeration. Plates were kept in a rice chamber under ambient light conditions (Photoperiod-16 hours of light and 8 hours of darkness, Temp- 20±1°C, and relative humidity- 65%). The germination parameters, including Mean Germination Time (MGT), Germination Index

(GI), and time taken to obtain 50% of germination (T_{50}), were calculated (Al-Mudaris 1998, Farooq et al., 2005, Kader 2005).

Emerging hypocotyls were observed every day, and the MGT was calculated:

$$MGT = \frac{\sum(nT)}{\sum n}$$

Where, n= number of germinated seeds at time T; T = Time from the beginning of the experiment to each germination count; $\sum n$ = Total number of germinated seeds

$$GI = (10 \times n_1) + (9 \times n_2) + (8 \times n_3) + \dots + (1 \times n_{10})$$

where $n_1, n_2, n_3, \dots$ are the number of seeds germinated on 1st, 2nd, 3rd day subsequently. The 10, 9, ..., 1 serve as a weighing factor corresponding to each day of observation.

$$T_{50} = t_i + \left(\frac{\frac{N}{2} - n_i}{n_j - n_i} \right) \times (t_j - t_i)$$

where, N is the final number of germinated seeds, n_i and n_j are the cumulative numbers of seeds germinated at times t_i and t_j , respectively, such that $n_i < N/2 < n_j$.

2.5. Greenhouse evaluation of seed treatment

The germinated seeds from the previous experiment were planted in the plastic pots, having a dimension of top diameter of 30.5 cm, bottom diameter of 17 cm, and height of 25.5 cm, filled with soil containing red soil, vermiculite, and vermicompost in the ratio 5:2:2. Then the pots were transferred to the glasshouse (16/8 photoperiod; Temp- $20 \pm 1^\circ\text{C}$, and relative humidity- 65%). The plant was maintained till the plants reached the yield stage. When the plants reached the yield stage, growth parameters such as root and shoot length, number of tillers, root volume, leaf width, fresh and dry weight, total chlorophyll, and yield were determined (Behera et al., 2023).

2.6. Defense suppression metrics in chitosan-primed seedlings

To assess the defence response of the chitosan-treated plant, the *R. solani* infection was introduced after 45 days after transplantation. *R. solani* was cultured in PDB (Potato Dextrose Broth) for 6 days at 27°C and 150 rpm. The resulting mycelial mats were harvested via centrifugation ($7000 \times g$, 20 min), washed and homogenized to prepare a mycelial suspension adjusted to 10^8 mycelial fragments/mL. Scissors were dipped in the fungal suspension for 5 mins to allow fragment attachment. Then the tip of the rice plant was clipped using the inoculated scissor to introduce the pathogen through a wound-mediated infection. Symptom progression was monitored daily.

Trichoderma viride (1 mg/mL) was sprayed onto the unprimed plants on alternate days, which served as the control.

2.6.1. Disease Severity Index (DSI)

DSI was observed daily and calculated using the formula (Chiang, Liu, Bock 2017).

$$DSI (\%) = \frac{[\sum(R \times N)]}{H \times T} \times 100$$

Where, R = disease scale difference (from scale 0-4); N = number of plant corresponding disease scale; H = highest scale category; T = total number of plants

scale; 0 = Healthy plant; 1 = Beginning of a yellowish green coloration; 2 = Yellowish-green coloration extension; 3 = Chlorotic, wilted, and brownish color leaves; 4 = Dead plants (Husna, Zakaria, Mohamed 2021).

2.6.2. Enzyme assay

The leaf tissue was harvested for antifungal enzymatic assay to study the defense response induced by priming of the seed. Approximately 200 mg of fresh leaves was homogenized with 80% chilled acetone and centrifuged at 7000×g for 10 mins. The supernatant was collected and used as the crude enzyme extract (Behera et al., 2023).

2.6.2.1. Chitinase enzyme assay

The chitinase enzyme assay was performed following Reissig et al., with minor modifications. Briefly, 200 µL of the crude enzyme was mixed with 200 µL of colloidal chitin substrate and incubated for 2 h. at 37°C. After incubation, the reaction mixture was centrifuged at 7000×g for 10 mins, and the resulting supernatant was collected. 50 µL of 0.8 M potassium tetraborate was added to the 200 µL of the supernatant and boiled for 3 mins. Subsequently, 1 mL of DMAB (DiMethyl Ammonium Bromide) was added and incubated till the solution developed a pink coloration. Absorbance was measured at 585 nm and compared to the N-acetylglucosamine standard curve. 1 Unit of chitinase enzyme activity is defined as the amount of enzyme that releases 1 µmol of N-acetylglucosamine per minute under the assay conditions (Reissig, Strominger, Leloir 1955).

2.6.2.2. Polyphenol oxidase (PPO) enzyme assay

The PPO enzyme assay was carried out according to Simões (Simões et al., 2015). The reaction solution comprised 1.4 mL of 15 mmol/L phosphate buffer (pH 7), 1.5 mL of 0.2 M catechol, and 50 µL of crude enzyme extract. Absorbance was taken at 425 nm for over 3min at 30 sec intervals. 1 Unit of PPO is defined as the amount of enzyme that causes an increase of 0.001 absorbance units per minute at 425 nm.

2.6.3. Gene Expression analysis

Total RNA was extracted from rice leaf tissues using the Trizol reagent method. The quality and concentration of RNA were assessed using a spectrophotometer. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a RT-PCR. Quantitative real-time PCR (qRT-PCR) was performed to analyze the expression levels of defense-related genes, including Pathogenesis-Related (PR) genes and Phenylalanine Ammonia-Lyase (PAL) gene. The relative gene expression was calculated using a housekeeping gene as an internal control. The primer used in the experiment is mentioned in Table 1.

Gene	Primer	Sequence
GAPDH	Forward	5' ATGGTCAGTCGAAACATTAC 3'
	Reverse	5' TCCTTTGTTCTTTTCCATCC 3'
PR1	Forward	5' CGTCTTCATCACCTGCAACTACTC 3'
	Reverse	5' CATGCATAAACACGTAGCATAGCA 3'
PR10	Forward	5' CCCTGCCGAATACGCCTAA 3'
	Reverse	5' CTCAAACGCCACGAGAATTTG 3'
PR1b	Forward	5' GTGGCGGAGAAGCAGTGGTA 3'
	Reverse	5' ACGGCTGACGGCTTTATTCC 5'
PAL/PR11	Forward	5' AGCACATCTTGGAGGGAAGCT 3'
	Reverse	5' GCGCGGATAACCTCAATTTG 3'

Table 1. The primer sequence used for the gene expression analysis of the plant samples

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons.

3. Result and Discussion

3.1. Effect of chitosan on germination efficiency

Seed priming is a well-established strategy to enhance germination efficiency, particularly under suboptimal environmental conditions (Farooq et al., 2005). The priming of seed improved the germination efficiency; these improvements can be attributed to enhanced water uptake, activation of early metabolic processes, and increased antioxidant activity induced by chitin derivative treatment (Behera et al., 2022). In the present study, IR64 rice seeds primed with both commercial and biologically synthesized chitosan demonstrated significantly improved germination performance compared to unprimed controls. Seeds treated with biosynthesized chitosan showed the most notable and uniform germination, exhibiting the lowest mean germination time (MGT $\sim$ 2.9 days) and shortest time to 50% germination (T_{50} $\sim$ 2.5 days). In comparison, seeds primed with commercial chitosan recorded an MGT of $\sim$ 3.5 days and a T_{50} of $\sim$ 3.1 days, while the unprimed control group showed the slowest germination (MGT of $\sim$ 4 days and T_{50} of $\sim$ 3.6 days). Although biosynthesized chitosan consistently displayed numerically superior germination metrics compared to commercial chitosan, statistical analysis ($p > 0.05$) indicated that these differences were not significant. Furthermore, both treatments markedly enhanced the germination index (GI), with biosynthesized chitosan yielding the highest value ($\sim$ 225), followed by commercial chitosan ($\sim$ 180), in contrast to the control ($\sim$ 110). Collectively, these findings suggest that chitosan priming improves seed vigour and metabolic activity during early growth (**Fig. 1**). The superior or comparable efficacy of biosynthesized chitosan may result from its unique structural properties, such as lower molecular weight, higher degree of deacetylation, and nano-scale features often determined by the microbial synthesis process (Badawy, Rebea 2011). Using

Streptomyces for chitosan production not only enhances its bioactivity but also adds a sustainable and eco-friendly dimension to its application in agriculture.

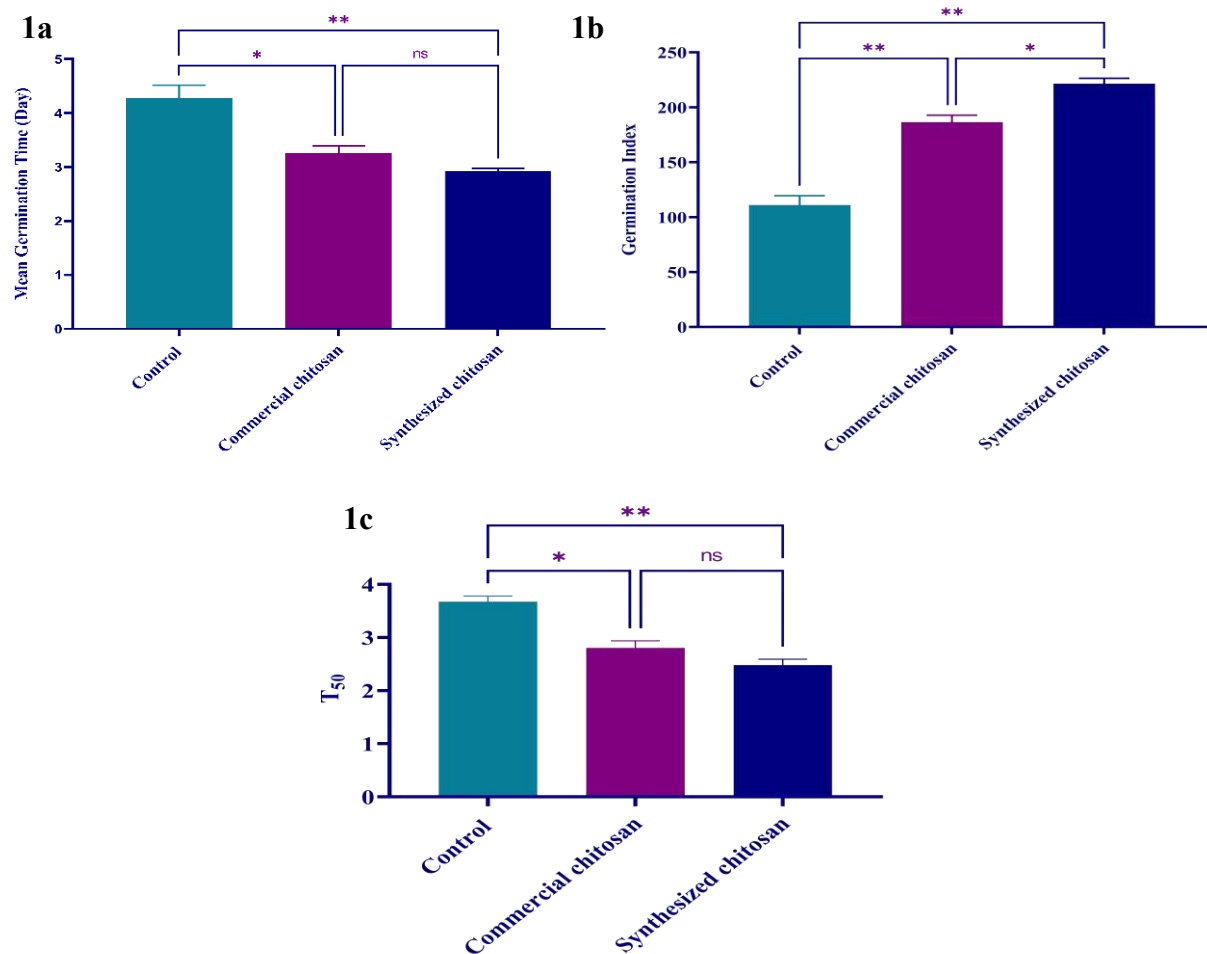


Fig. 1 The seed priming effect of chitosan on the germination parameters of IR64 rice periods: A) Mean germination time; B) Germination Index; C) T_{50} . The chitosan treatment showed better MGT, GI, and T_{50} as compared to all other groups. Though not significant, the synthesized chitosan showed better germination compared to the commercial counterpart. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

3.2. Effect of chitosan on plant growth promotion

3.2.1. Shoot length, root length, and root volume

The use of both commercial and synthesized chitosan was effective in enhancing root length, shoot length, and root volume compared to the control, with synthesized chitosan proving effective for all measured parameters (Supplementary Fig. 1 and 2). Plants treated with synthesized chitosan exhibited higher root elongation, i.e., 38.2 cm (Fig. 2a), and a greater shoot length of 75 cm (Fig. 2b), indicating a stronger growth response compared to

commercial treatment, which had a root length of 33.7 cm and a shoot length of 66 cm, while the control group had the lowest value with a root length of 24 cm and shoot length of 57 cm. The seed priming also increases the root volume from 173 mL in the control plant to 199.4 mL in commercial chitosan treatment and further to 227.5 mL in synthesized chitosan-treated plant (Fig. 2c). The increase in root and shoot growth also indicates the role of chitosan in shaping below-ground architecture and may be responsible for improved efficiency in nutrient and water uptake. Chitosan plays a role in modulating plant growth through signalling pathways related to auxins and other phytohormones, as well as by enhancing the availability of essential nutrients via its chelating properties. This conclusion is supported by research (Malreba and Cerana 2016), which noted that lower molecular weight chitosan and nanoparticulate forms show better bioavailability and effectiveness in promoting plant growth responses.

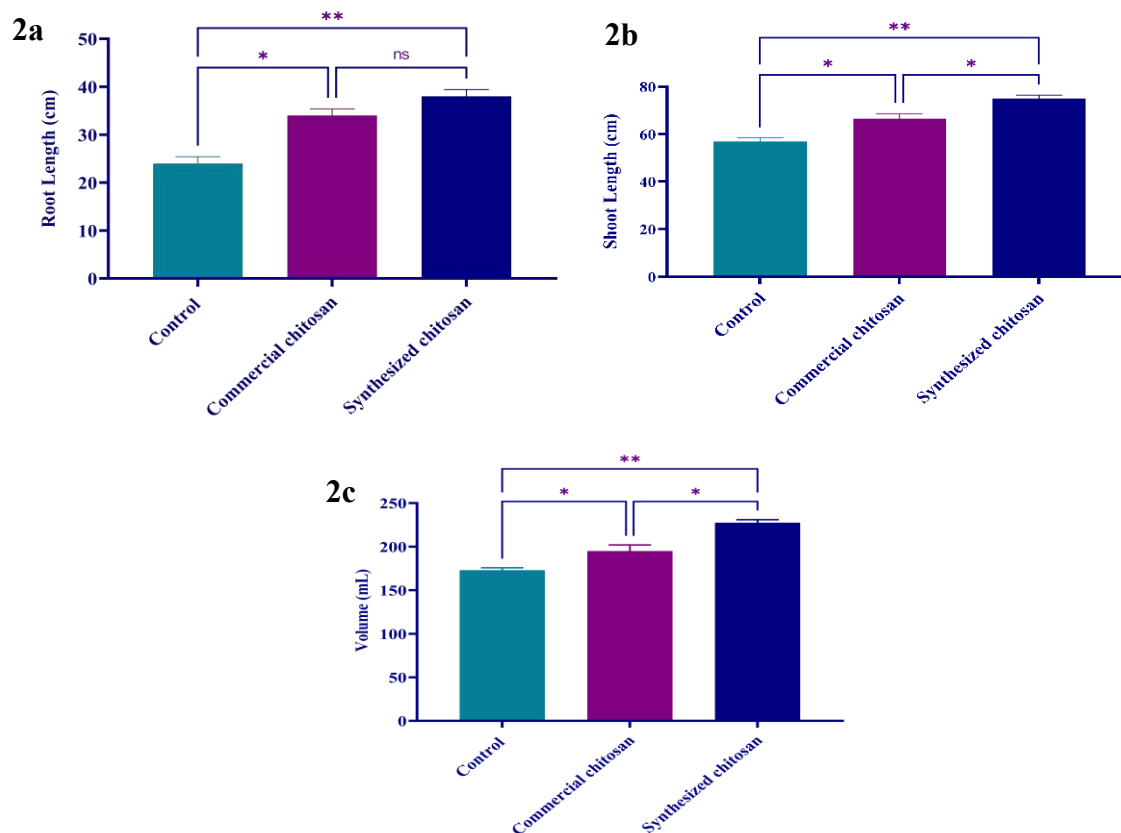


Fig. 2. The seed priming effect of chitosan on plant growth promotion parameters: A) Root length; B) Shoot length; C) Root volume. The chitosan treatment showed better root length, shoot length, and root volume as compared to all other groups. Though not significant, the synthesized chitosan showed better growth parameters compared to the commercial counterpart. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

3.2.2. Leaf width and No. of tillers

Commercial and synthesized chitosan treatment effectively stimulates vegetative rice growth, as indicated by significant increases in tiller count and leaf width over the control. In particular, synthesized chitosan-treated plants had ~14–18 tillers per plant and leaf widths of around 2–2.5 cm, followed by commercial chitosan counterpart with ~15 tillers and a leaf width of ~2 cm. Whereas the controls had only ~6 tillers and leaf widths of around 0.6 cm (**Fig. 3**). Notably, commercial and synthesized versions showed no significant disparity, implying that locally synthesized chitosan is a similarly good and possibly more cost-effective option. The ability to produce more tillers is an important indicator of potential grain yield in cereal plants, reflecting improved nutrient acquisition and hormonal regulation. Regarding leaf width, a broader leaf blade provides more photosynthetic surface area, leading to better energy assimilation and overall biomass production. These findings are reinforced by recent studies. Onumah observed that foliar application of low molecular weight chitosan in Ghana's Sudan Savanna significantly boosted rice growth and yield, highlighting its broad agronomic potential (Onumah et al., 2025). Similarly, Elshayb et al. (2024) demonstrated that using chitosan-based NPK nanostructures allowed a 30% reduction in synthetic fertilizer use without decreasing rice yields, showing chitosan's role in sustainable nutrient management (Elshayb et al., 2024).

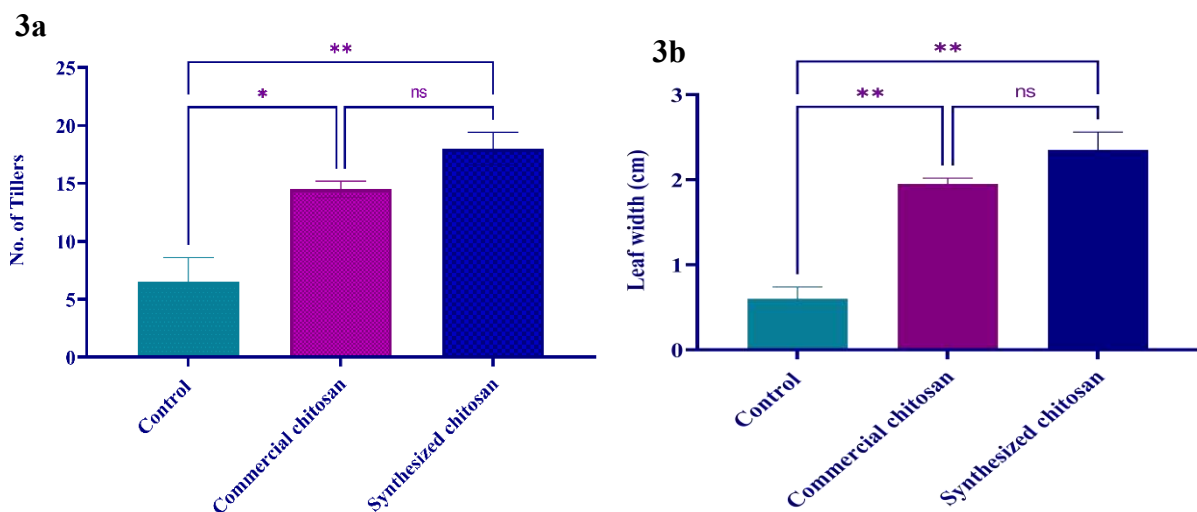


Fig. 3. Effect of seed priming of chitosan on: A) Number of tillers; B) Leaf width. The chitosan treatment showed better tiller and leaf width as compared to all other groups. Though not significant, the synthesized chitosan showed better growth parameters compared to the commercial counterpart. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

3.2.3. Biomass accumulation

Commercial and synthesized chitosan applications resulted in a significant increase in rice plant biomass accumulation. The fresh weight rose from about 45.2 g for control plants to 54.1 g for commercial chitosan-treated plants and 61.4 g for synthesized chitosan-treated plants. Dry weight too rose from 26.8 g (control) to 36.5 g (commercial chitosan) and 40.9 g (synthesized chitosan) (**Supplementary Fig. 3**).

The increase in plant biomass results from various physiological mechanisms triggered by chitosan. It has been shown to stimulate cell division and elongation, leading to organ expansion and tissue development. Additionally, it enhances nutrient uptake efficiency by regulating root architecture and increasing root surface area, which promotes better absorption of macro- and micronutrients (Riseh, Vazvani, Kennedy 2023). Moreover, chitosan influences stomatal regulation and chlorophyll biosynthesis, thereby improving photosynthetic efficiency and carbon fixation, which directly contributes to increased biomass (Elshayb et al., 2024). The rise in dry weight signifies not just greater water retention (as shown by fresh weight) but also actual improvements in the structural and metabolic content of plant tissues, likely due to enhanced nitrogen assimilation and stimulation of secondary metabolite pathways that support cell wall biosynthesis.

3.3. Physiological and Reproductive Traits

Total chlorophyll and grain yield increased considerably upon the application of chitosan. Total chlorophyll increased from 29.4 mg/L in the control to 36.7 mg/L using commercial chitosan and to 47.9 mg/L using synthesized chitosan. At the same time, grain yield increased from 12.4 g/plant for the control to 19.3 g/plant when using commercial chitosan and to 21.0 g/plant when using synthesized chitosan (**Fig. 4; Supplementary Fig. 4**). The increased chlorophyll level under chitosan treatment would most probably be a result of improved photosynthetic efficiency and postponed senescence, fueled by the elicitor activity of chitosan. Chitosan can induce genes for chlorophyll biosynthesis and maintain the structural integrity of the chloroplast, thereby ultimately enhancing light capture and energy conversion by the plant (Balusamy et al., 2022; Cao et al., 2025). In addition, chitosan has been found to enhance stomatal conductance and nutrient acquisition, particularly nitrogen and magnesium, which are essential for chlorophyll metabolism and photosynthesis (Balusamy et al., 2022). Higher biomass and yield noted may also be due to enhanced carbon fixation and stronger root structure promoted by chitosan treatment. Such physiological improvements enable increased translocation of photosynthates to developing grains, which results in heavier grain.

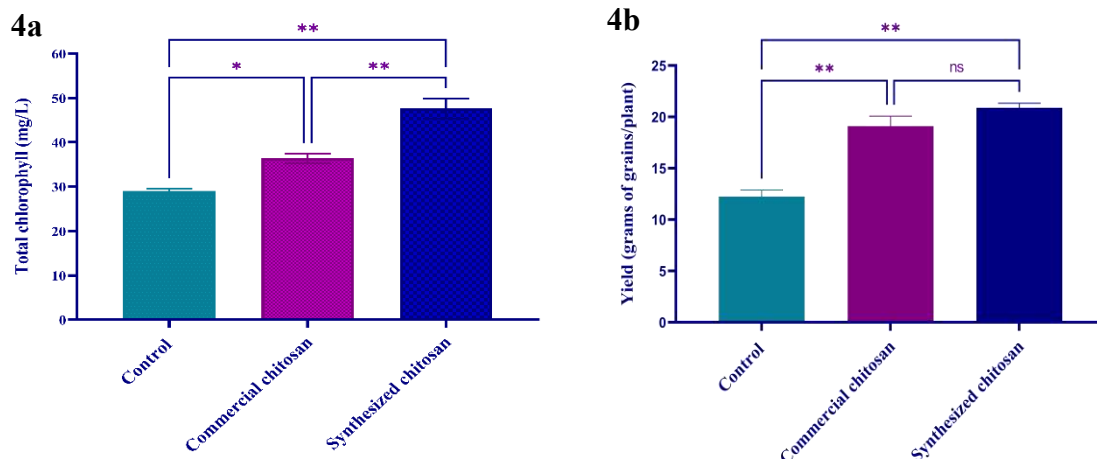


Fig. 4. Effect of chitosan on A) Total chlorophyll; B) Yield of rice plant. The chitosan treatment showed better chlorophyll content and Yield as compared to all other groups. Though not significant, the synthesized chitosan showed better yield compared to the commercial counterpart. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

3.4. Antifungal Activity

The DSI test showed that *Rhizoctonia solani*-infected rice plants had a considerable decrease in disease symptoms when treated with synthesized chitosan (**Fig. 5a**). The DSI value of the synthesized chitosan-treated group was the lowest among all the groups infected by pathogens, i.e. 7.83%. Meanwhile, commercial chitosan-treated plants showed partial suppression of disease, showing a DSI of 17.3% and fungicide-treated plants showed only symptomatic partial alleviation (DSI- 74.5%). Such findings indicate that synthesized chitosan significantly suppresses disease development and shields rice plants against *R. solani*-inflicted damage. It is also noteworthy that the efficacy of the fungicide was low, which could imply that the fungicide directly inhibits the fungal pathogen in a manner whereby little or no significant plant-mediated defense is triggered (**Supplementary Fig. 5**).

To better elucidate the defense mechanisms, the levels of various enzymes and PR genes were tested. It was observed that the seeds primed with chitosan have a better antifungal enzyme activity (chitinase and PPO activity) as compared to the untreated and fungicide treatment groups. Chitinase activity was found to be highest in the synthesized chitosan-treated plants (16.93 U/mg), followed by commercial chitosan (14.36 U/mg), and fungicide (10.12 U/mg), while the control plants showed minimal activity (3.98 U/mg). Similarly, PPO activity increased significantly in chitosan-primed plants, with synthesized chitosan reaching 18.67 U/mg, commercial chitosan 15.33 U/mg, compared to 10.16 U/mg in the fungicide group and 4.31 U/mg in the control (**Fig. 5b, 5c**). The chitosan treatment enhances the enzyme content by six times as compared to the untreated control.

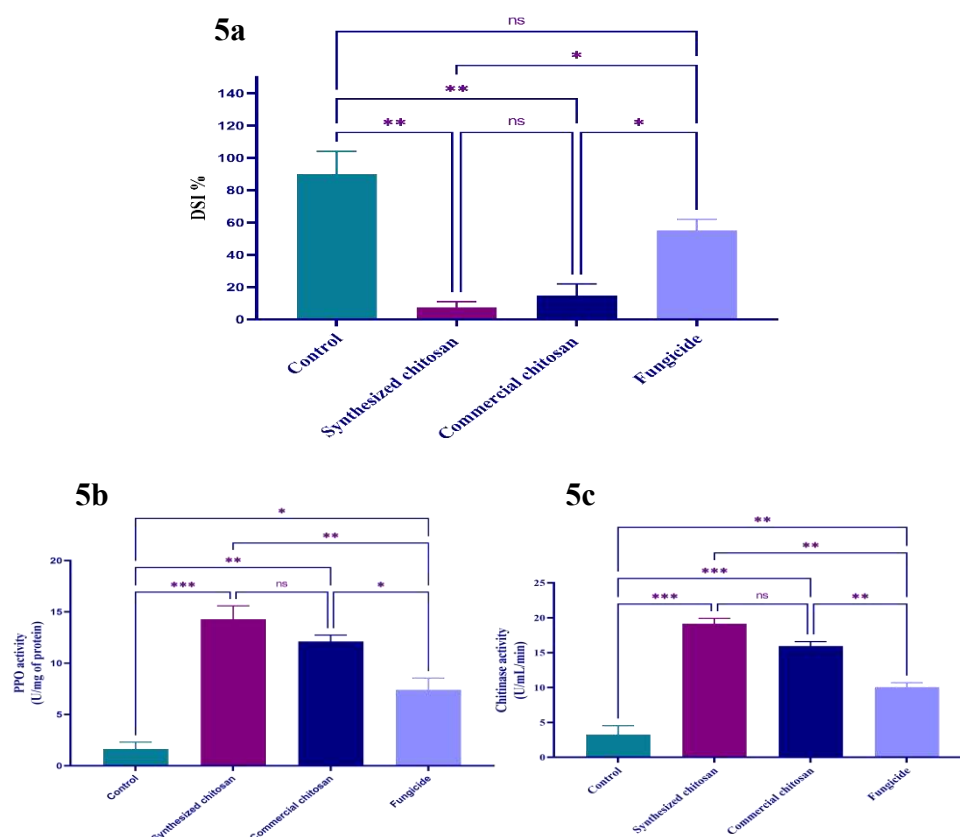


Fig. 5. Disease severity index and antifungal enzyme assay analysis of the rice plant after the treatment of chitosan on *R. solani* infection. The chitosan treatment showed less DSI and higher antifungal activity (chitinase and PPO activity) as compared to all other groups. Though not significant, the synthesized chitosan showed less DSI and higher chitinase and PPO activity compared to the commercial counterpart. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

In the gene expression analysis, only the PR10 gene was expressed (**Fig. 6**). Treatment with synthesized chitosan caused robust induction of antifungal enzyme activity and PR10 expression, which resulted in ~5-fold and ~70-fold more expression than the fungus-alone control, respectively, and are higher than that induced by commercial chitosan. The antifungal activity of chitosan suggests that chitosan not only has direct antifungal action but also induces systemic resistance mechanisms in rice, especially through the overexpression of enzymes like chitinase, polyphenol oxidase, and PR10 gene. Chitosan has polycationic properties, which allow it to interact with negatively charged material in the fungal cell membrane, resulting in enhanced permeability, loss of cellular contents, and eventually cell death. Moreover, chitosan can affect nutrient uptake and hamper spore germination and elongation of fungi (Palma-Guerrero et al., 2010). The biphasic mechanism of action, bridging inhibition of the pathogen and activation of the host defence, is in line with previous research establishing chitosan as both an antimicrobial compound and a plant immune elicitor (Rabea et al., 2003; El Hadrami et al., 2010). Thus, while fungicides might suppress the pathogen physically, chitosan-based treatments provide a more durable and sustainable approach by strengthening the plant's innate immune system in addition to direct pathogen management.

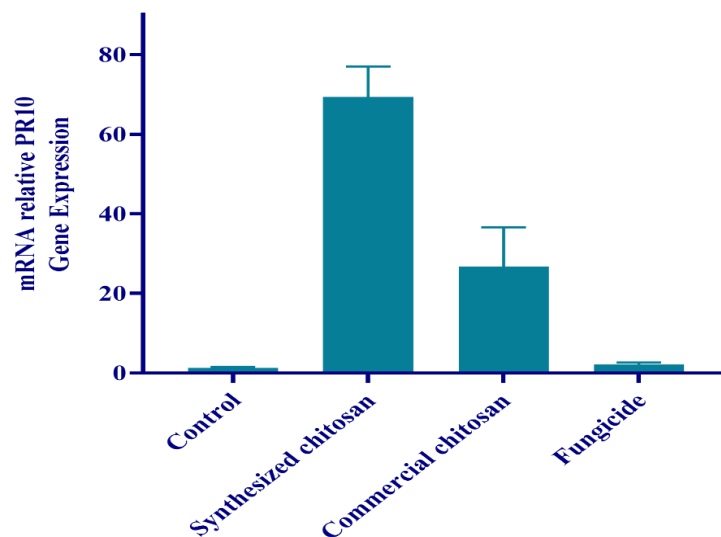


Fig. 6 Gene expression analysis of the rice plant after the treatment of chitosan on *R. solani* infection. The synthesized chitosan treatment showed 70 times more PR10 gene expression as compared to the control. Data were expressed as mean $\pm$ standard deviation (SD), and differences between treatment groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. Statistical significance was determined at $p \leq 0.05$. The

following notation was used to indicate significance levels: *ns* (not significant) for $p > 0.05$, $p = 0.03$ as *, $p = 0.0021$ as **, and $p = 0.0002$ as ***

Notably, although the differences were not statistically significant, the overall trend mostly favoured the microbial-synthesized chitosan across multiple physiological and biochemical parameters, including significantly higher PR10 gene expression. This could be due to its degree of deacetylation and its molecular weight, which are typically more uniform than the commercial chitosan, allowing plants to recognize it more efficiently through the receptors like CERK1 and CEBiP, leading to a stronger growth and defence response. Additionally, the microbial production of chitosan generates more defined oligomer fragments due to its specific enzymatic activity, while preserving the native functional groups, which enhances the solubility and receptor binding affinity, thereby amplifying downstream immune response (Hadwiger, Beckman 1980; Hadwiger, Ogawa, Kuyama 1994). This suggests that the synthesized chitosan can effectively replace its commercial counterpart, offering an eco-friendlier alternative that reduces the reliance on harsh chemical synthesis and aligns with sustainable agricultural practices.

5. Conclusion

In conclusion, chitosan produced from *Streptomyces* spp. was a promising candidate for a multifaceted bio-input in sustainable agriculture. It significantly improved plant growth by stimulating seed germination, root and shoot growth, and plant vigour. Simultaneously, microbial-synthesized chitosan significantly inhibited the growth of *Rhizoctonia solani* in vitro, possibly by breaking fungal cell membranes and increasing the expression of PR 10 gene. This two-way activity fostered plant growth and inhibited pathogen growth, which well illustrates the value of chitosan as a natural, environmentally friendly alternative to chemical fungicides and fertilisers. Large-scale field validation in the future, formulation development, and investigation of its synergistic action with beneficial soil microorganisms for integrated crop management practices would be the areas of investigation.

Conflict of Interest

The authors declare there is no potential conflict of interest among the authors.

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Author contribution

Subhransu Sekhar Behera, Lopamudra Ray: Conceptualization; Subhransu Sekhar Behera, Seemon Giri, Zahra Parwez, Smaranika Pradhan: Investigation and Methodology; Subhransu Sekhar Behera, Seemon Giri, Lopamudra Ray: Data curation; Subhransu Sekhar Behera, Seemon Giri, Lopamudra Ray: Formal analysis and Validation; Subhransu Sekhar Behera and Lopamudra Ray: Writing – original draft; Lopamudra Ray: Review & editing

Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

- Al-Mudaris M: Notes on Various Parameters Recording the Speed of Seed Germination. JARTS. 1998, 992:147–154.
- Badawy MEI, Rabea EI: A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection. International Journal of Carbohydrate Chemistry. 2011, 2011:460381. 10.1155/2011/460381.
- Balusamy SR, Rahimi S, Sukweenadhi J, et al: Chitosan, Chitosan Nanoparticles, and Modified Chitosan Biomaterials, a Potential Tool to Combat Salinity Stress in Plants. Carbohydrate Polymers. 2022, 284:119189. 10.1016/j.carbpol.2022.119189.
- Behera HT, Mojumdar A, Das S, et al: Exogenous N-Acetylated Chitooligosaccharides Application Improves Osmotic Stress and Water Deficit Tolerance in *Oryza sativa* (cv. Swarna Sub-1) Inducing Seed Germination, Seedling Growth, and Biochemical Activities. Plant Physiology Reports. 2022, 27:443–457. 10.1007/s40502-022-00680-x.
- Behera SS, Nivedita S, Behera HT, et al: Biosynthesis of Chitosan from Shrimp Shell Chitin Using *Streptomyces griseoincarnatus* RB7AG: Characterization and Application as Coating Material to Enhance Shelf Life of Tomatoes (Var. Ruby Pusa) in Post-Harvest Storage Condition. Bioactive Carbohydrates and Dietary Fibre. 2024, 32:100455. 10.1016/j.bcdf.2024.100455.
- Behera SS, Nivedita S, Behera PK, et al: *Streptomyces griseoincarnatus* Strain RB7AG Promotes *Oryza sativa* (Var. Swarna) Growth under Salt Stress: Mechanisms and Potential Applications. Letters in Applied Microbiology. 2023, 76:125. 10.1093/lambio/ovad125.
- Cao Y, Yan R, Sun M, et al: Effects of Exogenous Chitosan Concentrations on Photosynthesis and Functional Physiological Traits of Hibiscus under Salt Stress. BMC Plant Biology. 2025, 25:419. 10.1186/s12870-025-06424-x.
- Chiang KS, Liu HI, Bock C: A Discussion on Disease Severity Index Values. Part I: Warning on Inherent Errors and Suggestions to Maximise Accuracy. Annals of Applied Biology. 2017, 171:139–154. 10.1111/aab.12362.
- El Hadrami A, Adam LR, El Hadrami I, et al: Chitosan in Plant Protection. Marine Drugs. 2010, 8:968–987. 10.3390/md8040968.
- Elshayb OM, Ghazy HA, Wissa MT, et al: Chitosan-Based NPK Nanostructure for Reducing Synthetic NPK Fertilizers and Improving Rice Productivity and Nutritional Indices. Frontiers in Sustainable Food Systems. 2024, 8:1464021. 10.3389/fsufs.2024.1464021.
- Fahad S, Adnan M, Noor M, et al: Major Constraints for Global Rice Production. In *Advances in Rice Research for Abiotic Stress Tolerance*. 2019, 1:1–22. 10.1016/B978-0-12-814332-2.00001-0.
- Falcón-Rodríguez AB, Costales D, Rogers HJ, et al: Practical Use of Oligosaccharins in Agriculture. Acta Horticulturae. 2012, 1009:195–212. 10.17660/ActaHortic.2013.1009.24.

492 Farooq M, Basra SMA, Ahmad N, et al: Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice.
 493 Journal of Integrative Plant Biology. 2005, 47:187–193. 10.1111/j.1744-7909.2005.00031.x.

494 Hadwiger LA, Beckman JM: Chitosan as a Component of Pea-Fusarium solani Interactions. Plant Physiology.
 495 1980, 66:205–211. 10.1104/pp.66.2.205.

496 Hadwiger LA, Ogawa T, Kuyama H: Chitosan Polymer Sizes Effective in Inducing Phytoalexin Accumulation
 497 and Fungal Suppression Are Verified with Synthesized Oligomers. Molecular Plant-Microbe Interactions. 1994,
 498 7:531–533. 10.1094/mpmi-7-0531.

499 Hsu SC, Lockwood JL: Powdered Chitin Agar as a Selective Medium for Enumeration of Actinomycetes in Water
 500 and Soil. Applied Microbiology. 1975, 29:422–426. 10.1128/am.29.3.422-426.1975.

501 Husna A, Zakaria L, Mohamed Nor NM: Fusarium commune Associated with Wilt and Root Rot Disease in Rice.
 502 Plant Pathology. 2021, 70:123–132. 10.1111/ppa.13270.

503 Kader MA: A Comparison of Seed Germination Calculation Formulae and the Associated Interpretation of
 504 Resulting Data. Journal and Proceedings of the Royal Society of New South Wales. 2005, 138:65–75.
 505 10.5962/p.361564.

506 Lamichhane JR, Debaeke P, Steinberg C, et al: Abiotic and Biotic Factors Affecting Crop Seed Germination and
 507 Seedling Emergence: A Conceptual Framework. Plant and Soil. 2018, 432:1–28. 10.1007/s11104-018-3780-9.

508 Malerba M, Cerana R: Chitosan Effects on Plant Systems. International Journal of Molecular Sciences. 2016,
 509 17:996. 10.3390/ijms17070996.

510 Onumah J, Boamah PO, Aduguba WO, et al: Effect of Foliar Application of Low Molecular Weight Chitosan on
 511 Rice Growth and Yield in the Sudan Savanna Zone of Ghana. Carbohydrate Polymer Technologies and
 512 Applications. 2025, 10:100753. 10.1016/j.carpta.2025.100753.

513 Palma-Guerrero J, Lopez-Jimenez JA, Pérez-Berná AJ, et al: Membrane Fluidity Determines Sensitivity of
 514 Filamentous Fungi to Chitosan. Molecular Microbiology. 2010, 75:1021–1032. 10.1111/j.1365-
 515 2958.2009.07039.x.

516 Rabea EI, Badawy MET, Stevens CV, et al: Chitosan as Antimicrobial Agent: Applications and Mode of Action.
 517 Biomacromolecules. 2003, 4:1457–1465. 10.1021/bm034130m.

518 Reissig JL, Strominger JL, Leloir LF: A Modified Colorimetric Method for the Estimation of N-Acetylamino
 519 Sugars. Journal of Biological Chemistry. 1955, 217:959–966. 10.1016/S0021-9258(18)65959-9.

520 Riseh RS, Vazvani MG, Kennedy JF: The Application of Chitosan as a Carrier for Fertilizer: A Review.
 521 International Journal of Biological Macromolecules. 2023, 252:126483. 10.1016/j.ijbiomac.2023.126483.

522 Samal I, Bhoi TK, Vyas V, et al: Resistance to Fungicides in Entomopathogenic Fungi: Underlying Mechanisms,
 523 Consequences, and Opportunities for Progress. Tropical Plant Pathology. 2024, 49:5–17. 10.1007/s40858-023-
 524 00585-6.

525 Savvides A, Ali S, Tester M, et al: Chemical Priming of Plants against Multiple Abiotic Stresses: Mission Possible?
526 Trends in Plant Science. 2016, 21:329–340. 10.1016/j.tplants.2015.11.003.

527 Senapati M, Tiwari A, Sharma N, et al: Rhizoctonia solani Kühn Pathophysiology: Status and Prospects of Sheath
528 Blight Disease Management in Rice. Frontiers in Plant Science. 2022, 13:881116. 10.3389/fpls.2022.881116.

529 Simões AND, Moreira SI, Mosquim PR, et al: The Effects of Storage Temperature on the Quality and Phenolic
530 Metabolism of Whole and Minimally Processed Kale Leaves. Acta Scientiarum Agronomy. 2015, 37:101–107.
531 10.4025/actasciagron.v37i1.18123.

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