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IN VITRO EVALUATION OF ANTIFUNGAL ACTIVITY OF FUNGAL-DERIVED CHITOSAN AGAINST *RHIZOCTONIA SOLANI*: A SUSTAINABLE APPROACH TO SOIL-BORNE DISEASE MANAGEMENT

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ABSTRACT

Fungal-derived chitosan has emerged as a sustainable and biologically potent alternative to conventional fungicides, offering eco-friendly advantages and broad-spectrum antimicrobial activity. Extracted from *Trichoderma viride*, this biopolymer not only aligns with organic and vegan agricultural practices but also capitalizes on the innate antagonistic properties of its fungal source. In the present study, the antifungal efficacy of purified fungal chitosan was evaluated in vitro against *R. solani* Kuhn, a notorious soil-borne pathogen responsible for damping-off and root diseases across various crops.Chitosan solutions ranging from 100 to 3000 ppm were tested using the poisoned food technique. The results demonstrated a clear concentration-dependent inhibition of *R. solani* mycelial growth. While negligible inhibition (0.39%) was observed at 100 ppm, higher concentrations significantly suppressed fungal growth, with complete inhibition (100%) achieved at 3000 ppm. The findings were statistically indicating the reliability of the assay.The antifungal mechanism of chitosan is attributed to its polycationic structure, which disrupts fungal membrane integrity, chelates essential nutrients, and elicits host plant immune responses. Compared to crustacean-sourced chitosan, fungal chitosan is less allergenic and more sustainable, with added bioactivity due to its origin. These in vitro results validate the potential of fungal chitosan as a natural disease management agent against *R. solani* and support its inclusion in integrated disease management strategies. Future investigations should focus on field applications and combinatorial use with other biological agents to enhance disease suppression in horticultural systems.

Keywords: Fungal chitosan, *Rhizoctonia solani*, Antifungal activity, Poisoned food technique, Sustainable biocontrol.

Introduction

Soil-borne pathogens like *R. solani* Kuhn represent a serious constraint in crop production worldwide, owing to their ability to cause severe damping-off, root rot, and collar rot in a wide variety of economically important crops. *R. solani* is a necrotrophic fungal pathogen with a broad host range and is particularly notorious in horticultural and vegetable cropping systems (Sneh *et al*., 1996). Its saprophytic survival ability through sclerotia or mycelia in soil, combined with its aggressive pathogenicity, poses a challenge to disease management under conventional systems (Lakshman *et al*., 2002).

Conventional management strategies rely heavily on synthetic fungicides, which have raised concerns over resistance development, phytotoxicity, environmental pollution, and residual toxicity in soil and crops (Rabea *et al*., 2009; Chaudhary *et al.,* 2020). As a result, there is an increasing emphasis on identifying eco-friendly alternatives for plant protection. Among these, chitosan has emerged as a versatile biopolymer with antimicrobial and plant-defense-inducing properties. Chitosan is obtained from the partial deacetylation of chitin, a natural polymer abundantly found in the exoskeletons of crustaceans and cell walls of fungi (Rinaudo, 2006).

In recent years, fungal-sourced chitosan has gained attention due to its vegan-compatible origin, consistent quality, and compatibility with sustainable and organic agricultural practices (Pochanavanich & Suntornsuk, 2002; Crognale *et al*., 2022). The use of fungal chitosan, especially derived from *T. viride*, offers the added advantage of leveraging the biological control traits of the source organism (Harman *et al*., 2004).

Chitosan exerts its antifungal action through multiple mechanisms: altering fungal membrane integrity, chelating vital nutrients, and eliciting host immune responses such as production of PR-proteins, phytoalexins, and reactive oxygen species (El Hadrami *et al.,* 2010; Xing *et al*., 2016). Although substantial studies have explored the effects of crustacean chitosan on *R. solani*, data regarding fungal-derived chitosan are still limited. Therefore, this study was undertaken to evaluate the in vitro antifungal activity of purified fungal chitosan extracted from *T. viride* against *R. solani* using the poisoned food technique.

Materials and Methods

Preparation of Chitosan Solution Purified fungal chitosan was dissolved in 1.0 per cent (v/v) acetic acid. A stock solution of 3000 ppm was prepared by dissolving 0.3 g chitosan in 100 ml of acetic acid. The pH of the solution was adjusted to 5.6 and autoclaved at 121°C for 20 minutes under 15 lbs pressure. Working concentrations of 100, 500, 1000, 1500, 2000, 2500, and 3000 ppm were prepared from the stock.

Fungal Pathogen and Bioassay Procedure

The virulent isolate of *R. solani* was obtained from the Department of Plant Pathology, N.M.C.A., NAU, Navsari, and maintained on PDA medium. The antifungal activity was evaluated using the poisoned food technique. PDA was amended with various chitosan concentrations before solidification and poured into Petri plates. A 5 mm disc of *R. solani* (7-day-old culture) was placed at the center of each plate. Control plates contained PDA without chitosan.

The plates were incubated at 27°C for 5–7 days until the control mycelia reached the edge of the plate. Radial growth was measured, and percent inhibition over control was calculated using the formula:

$$\% \text{ inhibition} = [(dc - dt) / dc] \times 100$$

Where, dc = radial growth in control,

dt = radial growth in treated plates.

Statistical Analysis

The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Standard Error (SEm ±), Critical Difference (CD at P=0.01), and Coefficient of Variation (CV%) were computed.

Results

The *in vitro* bioassay demonstrated a strong, concentration-dependent antifungal response of fungal chitosan against *R. solani*. At the lowest concentration of 100 ppm, minimal inhibition (0.39%) was observed. As the concentration increased, there was a consistent and statistically significant rise in the inhibition percentage. At 500 ppm, the inhibition rose to 20.39%, and further increased to 33.72% at 1000 ppm. Substantial inhibition was recorded at 1500 ppm (41.17%) and 2000 ppm (56.86%). At 2500 ppm, the inhibition reached 65.88%, and complete growth suppression (100%) was observed at the highest concentration of 3000 ppm (Fig 1 and Fig 2). The increasing trend clearly indicates a dose-dependent antifungal effect.

These observations were statistically validated, with a critical difference (CD) at 1% level of 4.47, and a coefficient of variation (CV) of 4.71%, indicating high precision and reliability of the experimental results. The findings are consistent with previous reports on chitosan's mode of action and its dose-related efficacy against phytopathogenic fungi. The summarized data are presented in Table 1.

Discussion

The present investigation confirms the strong antifungal potential of fungal-derived chitosan against *R. solani*, exhibiting a clear concentration-dependent trend. The lack of substantial inhibition at the lowest concentration (100 ppm) aligns with reports by Rubina *et al*. (2017) and Ahmed *et al*. (2019), where sub-lethal doses failed to suppress fungal growth. However, as the concentration increased, the antifungal action became more pronounced, with complete inhibition achieved at 3000 ppm. This confirms the hypothesis that a threshold concentration of chitosan is essential for disrupting fungal physiology.

Mechanistically, the antifungal effect of chitosan is attributed to its polycationic nature, enabling interaction with the anionic components of the fungal cell wall and membrane, thereby compromising membrane integrity (Badawy & Rabea, 2011; Xing *et al*., 2016). This leads to cytoplasmic leakage, inhibition of enzymatic functions, and eventual cell death. Liu *et al*. (2001) reported similar findings, emphasizing chitosan-induced leakage of intracellular electrolytes from fungal cells.

The chelation of essential metal ions such as Zn, Mg, and Ca by chitosan further disrupts fungal metabolism by inhibiting vital enzymatic pathways (Crognale *et al*., 2022). Moreover, El Hadrami *et al.* (2010) emphasized chitosan's role in eliciting plant immune responses, including enhanced synthesis of PR proteins and oxidative bursts, which could provide synergistic protection under field conditions. The complete inhibition observed at 3000 ppm is consistent with earlier studies by Zeitar *et al*. (2023), who demonstrated strong suppression of soil-borne fungi by chitosan at high concentrations. Similarly, Kalagatur *et al*. (2018) encapsulated essential oils in chitosan nanoparticles and observed potent inhibition of fungal pathogens, highlighting the carrier potential of chitosan.

The use of fungal chitosan offers several advantages over crustacean chitosan. It is compatible with organic and vegan farming, and its extraction is considered more sustainable and less allergenic (Pochanavanich & Suntornsuk, 2002; Kumari *et al*., 2021). Moreover, the source organism *T. viride* is itself a known antagonist of *R. solani* due to its production of cell wall-degrading enzymes (Harman *et al.,* 2004).

Though *in vitro* results may not fully replicate field scenarios, the promising inhibition observed in this study justifies further research under greenhouse and field conditions. Additionally, optimization of molecular weight and degree of deacetylation of chitosan, as suggested by Rinaudo (2006) and Younes & Rinaudo (2015), may further enhance its bio efficacy.

Conclusion

This study demonstrated that fungal chitosan derived from *T. viride* possesses significant antifungal activity against *R. solani* in vitro. The antifungal effect was clearly concentration-dependent, with complete inhibition achieved at 3000 ppm. These findings highlight the potential of fungal chitosan as an eco-friendly alternative to synthetic fungicides for managing soil-borne pathogens. Future studies should explore chitosan's performance under field conditions and in combination with microbial consortia or plant resistance inducers to enhance its efficacy as part of an integrated disease management strategy.

Table: 1 *In vitro* evaluation of fungal chitosan against *R. solani*

Tr. No	Chitosan Concentration (ppm)	Per cent inhibition over control *Rhizoctonia solani*
T1	**100**	0.39
T2	**500**	20.39
T3	**1000**	33.72
T4	**1500**	41.17
T5	**2000**	56.86
T6	**2500**	65.88
T7	**3000**	100.00
T8	**Control**	0.00
	SE m±	1.08
	CD (P=0.01)	4.47
	CV (%)	4.71

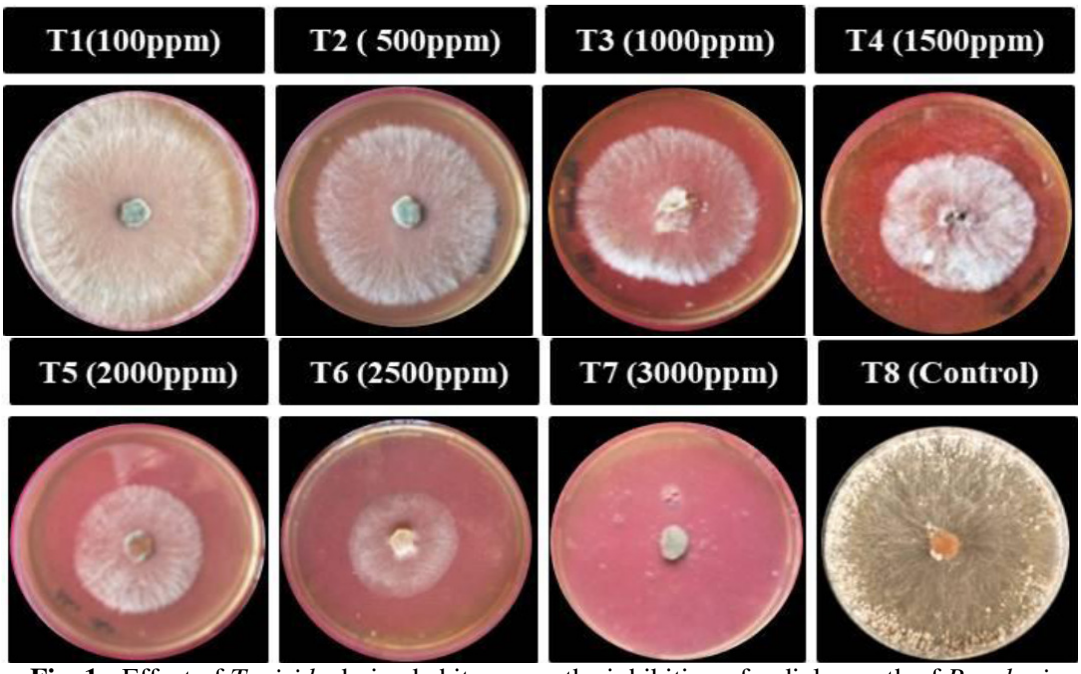

Fig. 1 : Effect of *T. viride* derived chitosan on the inhibition of radial growth of *R. solani*

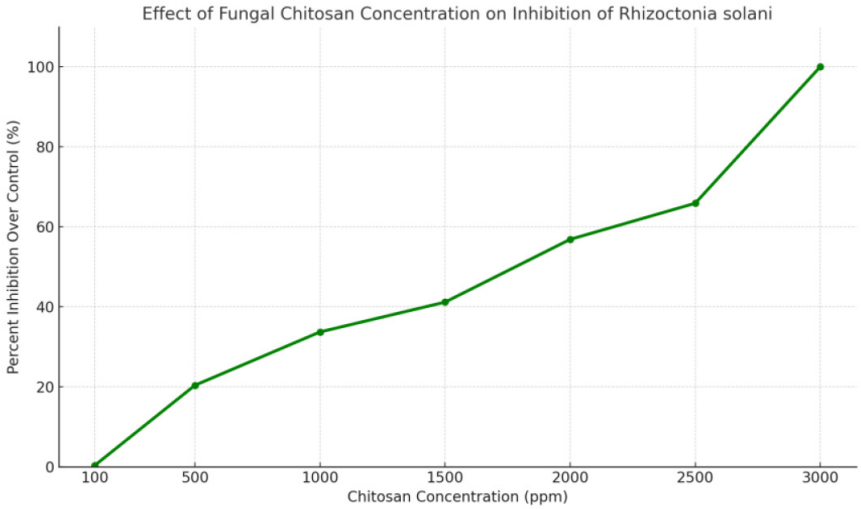

Fig. 2 : Effect of *T. viride* derived chitosan on the inhibition of radial growth of *R. solani*

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Conflict of interest

The authors declare that they have no knows competing financial interests or personal relationship that could have approved to influence the work reported in this paper.

Ethical statement

All the experimental procedures involving only on fungal species were conducted following the N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India institutional guidelines. There are no human and animal subjects/trials conducted in this article and informed consent is not applicable.

Disclosure statement

The authors declare that there are no financial/ commercial conflicts of interest.

Author contributions

Divya shree (Conceptualization [supporting], Data curation [lead], Formal analysis [lead], Investigation [lead], Visualization [lead], Writing –original draft [lead]), Lalit Mahatma (Supervision [supporting], Validation [equal], Writing –review & editing [equal]), B. Anil Kumar & T. L. Shivananda (Data curation [Equal]).

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