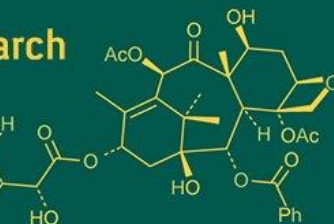
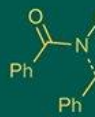


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Mahima J

PG Scholar, Plant Pathology
and Agricultural Microbiology
Section, Govt. College of
Agriculture, Pune,
Maharashtra, India

Lohate SR

Junior Pathologist, AICRP on
Floriculture, Zonal
Agricultural Research Station,
Ganeshkhind, Pune,
Maharashtra, India

Dalvi SG

Senior Scientist, Tissue Culture
Agriculture Science and
Technology, Vasantdada Sugar
Institute, Manjari (Bk.), Pune,
Maharashtra, India

Karade VM

Professor of Plant Pathology,
Govt. College of Agriculture,
Pune, Maharashtra, India

Chavanke SN

Scientific Officer, Tissue
Culture Agriculture Science
and Technology, Vasantdada
Sugar Institute, Manjari (Bk.),
Pune, Maharashtra, India

Lubna Ghajal

PG Scholar, Plant Pathology
and Agricultural Microbiology
Section, Govt. College of
Agriculture, Pune,
Maharashtra, India

Corresponding Author:**Mahima J**

PG Scholar, Plant Pathology
and Agricultural Microbiology
Section, Govt. College of
Agriculture, Pune,
Maharashtra, India

***In vitro* evaluation of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate against *Sclerotium rolfii* causing tuberose stem rot**

Mahima J, Lohate SR, Dalvi SG, Karade VM, Chavanke SN and Lubna Ghajal

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Abstract

Tuberose (*Agave amica*) is an economically important ornamental crop increasingly constrained by stem rot caused by *Sclerotium rolfii*. This study evaluated the *in vitro* antifungal potential of chitosan nanoparticles, β -glucan nanoparticles and chitosan-copper- β -glucan nanoconjugate against *S. rolfii* isolated from diseased tuberose stems. Antifungal efficacy was assessed by the poisoned food technique across a concentration gradient using mycelial growth inhibition and sclerotial germination as complementary endpoints. Chitosan nanoparticles and β -glucan nanoparticles exhibited pronounced concentration-dependent activity, achieving maximum mycelial growth inhibition of 97.41% and 97.04%, respectively, at 700 ppm. Notably, chitosan-copper- β -glucan nanoconjugate produced 98.15% inhibition at only 300 ppm and completely suppressed sclerotial germination at the same concentration, compared with 100% germination in the untreated control. Thus, the nanoconjugate achieved near-complete mycelial suppression at less than half the concentration required for the individual nanoparticles, while simultaneously targeting the pathogen's persistent survival structure. These findings identify chitosan-copper- β -glucan nanoconjugate as the most potent nanomaterial evaluated and demonstrate its strong *in vitro* potential as a nano-enabled strategy for suppression of *S. rolfii*. The results provide a compelling basis for subsequent greenhouse and field validation of this nanocomposite for sustainable management of tuberose stem rot.

Keywords: Chitosan, β -glucan, chitosan-copper- β -glucan Nanoconjugate, *Sclerotium rolfii*, tuberose, Sclerotial germination

1. Introduction

Medicinal plants have greatly gained importance in the management and treatment of human diseases, globally [1]. It constitutes the main source of primary healthcare in most rural populations, especially in many African countries, due to its affordability and availability. However, as the usage of these medicinal plants becomes more widespread around the world, there are questions and misgivings about the quality, efficacy, and safety of the products utilized in the health sector [2, 3, 4]. These concerns have hampered the sensible use and validation of Tuberose (*Agave amica*) is an important commercial ornamental crop valued for its fragrant white flowers and its use in loose flowers, garlands, floral arrangements and perfume production [1, 2]. However, its production is affected by several fungal diseases, among which stem rot caused by *S. rolfii* is an important constraint, particularly under warm and humid conditions [3, 4]. The pathogen produces persistent sclerotia that serve as important survival structures and contribute to disease recurrence [5, 6].

Management of *S. rolfii* mainly relies on chemical fungicides and biological control agents, but limitations associated with repeated chemical application and variable biocontrol performance have encouraged the search for alternative approaches [7, 8]. Chitosan and β -glucans are biologically active polysaccharides capable of interacting with plant defence systems and chitosan can function both as a direct antimicrobial compound and as an elicitor of plant defence responses. Chitin and β -glucan-derived molecular patterns can participate in plant immune recognition, while chitosan has been associated with activation of defence-related responses and secondary metabolism [9, 10]. Incorporation of copper into chitosan-based formulations may further enhance antimicrobial activity [11, 12].

Therefore, a chitosan-copper- β -glucan-based nanosystem may provide an opportunity to combine direct antifungal activity with host-mediated disease resistance.

Recent studies have demonstrated the antifungal potential of chitosan-based nanocomposites against *S. rolfsii* [13, 14]. Chavanke and Dalvi [15] also reported enhanced antifungal activity of a chitosan-copper- β -glucan nanocomposite against *S. rolfsii*. However, comparative evaluation of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate against *S. rolfsii* strain causing tuberose stem rot, particularly for mycelial growth and sclerotial germination, remains limited. Therefore, the present study was undertaken to evaluate their *in vitro* antifungal activity against *S. rolfsii*.

2. Materials and Methods

The present investigation entitled “*In vitro* evaluation of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate against tuberose stem rot (*Sclerotium rolfsii*)” was carried out during 2025-2026 in the Department of Plant Pathology, Govt. College of Agriculture, Pune, Maharashtra, India. The well-characterized nanomaterials used in the study were obtained from Vasantdada Sugar Institute (VSI), Manjari (Bk.), Pune, Maharashtra, India [15]. The stock concentrations provided were 3000 ppm each for chitosan and β -glucan and 2000 ppm for CN-Cu-BG nanoconjugate.

2.1 Characterization of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate

The physicochemical characteristics of these nanomaterials have been previously reported by Chavanke and Dalvi [10, 15]. Chitosan and β -glucan nanoparticles showed nanoscale particle sizes, with average zeta-size sizes of 64.43 nm for chitosan and 60.16-73.66 nm for β -glucan nanoparticles [10]. The CN-Cu-BG nanoconjugate exhibited a uniformly spherical morphology with an average particle size of 41.97 nm and spectroscopic and elemental analyses confirmed the incorporation of copper within the chitosan- β -glucan matrix [15]. These previously reported characteristics confirm the nanoscale nature of the nanomaterials used in the present investigation.

2.2 Isolation and identification of the pathogen

Diseased samples of tuberose showing typical stem rot symptoms were collected from AICRP on Floriculture, ZARS, Ganeshkhind, Pune (18°33'44.7"N 73°49'30.4"E) and brought to the laboratory for isolation of the pathogen. The pathogen was isolated from infected stem tissues using the tissue isolation technique on potato dextrose agar (PDA). The emerging fungal growth was purified by hyphal-tip isolation and maintained on PDA. The isolate was identified based on cultural and morphological characteristics, including mycelial colour, texture, growth pattern and sclerotial characteristics and identified as *S. rolfsii*.

2.3 *in vitro* evaluation of antifungal activity

The antifungal activity of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate against *S. rolfsii* was evaluated using the poisoned food technique. The respective concentrations were incorporated into sterilized PDA medium and 20 mL of the amended medium was poured into sterile 90-mm Petri plates. PDA without nanomaterial

served as the untreated control. After solidification, a 5-mm mycelial disc from an actively growing culture of *S. rolfsii* was placed at the centre of each plate. The plates were incubated at 28 °C. Mycelial growth was observed for approximately 5-7 days and observations were recorded when the untreated control plate was completely covered by fungal growth. Colony diameter was measured in centimetres.

Chitosan and β -glucan were evaluated at 50, 100, 200, 300, 400, 500, 600 and 700 ppm, whereas the nanoconjugate was evaluated at 50, 100, 150, 200, 250 and 300 ppm, along with an untreated control. Each treatment was maintained in three replications.

2.4 Mycelial growth inhibition

The percentage inhibition of mycelial growth over the untreated control was calculated using the formula of Vincent [20]:

$$I = [(C-T)/C] \times 100$$

Where I = percent inhibition, C = mycelial growth in control (cm) and T = mycelial growth in treatment (cm).

The effect of the treatments on mycelial morphology was also recorded based on growth pattern and mycelial colour in comparison with the untreated control.

2.5 Sclerotial germination assay

The effect of the chitosan-copper- β -glucan nanoconjugate on sclerotial germination of *S. rolfsii* was evaluated at 50, 100, 150, 200, 250 and 300 ppm along with an untreated control. Five sclerotia were used per replicate and placed on the respective poisoned PDA medium. The plates were incubated at 28 °C for 72 h, after which sclerotial germination was recorded based on visible emergence of mycelium from the sclerotia. The percentage of sclerotial germination was calculated as:

$$\text{Sclerotial germination (\%)} = (\text{Number of germinated sclerotia} / \text{Total number of sclerotia}) \times 100$$

2.6 Experimental design and statistical analysis

The data recorded for the different observations were analyzed using Completely Randomized Design (CRD). Statistical analysis was carried out following the standard procedures described by Panse and Sukhatme [16]. The significance of treatment differences was tested using analysis of variance and treatment means were compared using the critical difference (C.D.) at the appropriate level of significance. The analysis was performed using the OPSTAT online statistical application.

3. Results

During the present investigation on the “*In vitro* evaluation of chitosan, β -glucan and chitosan-copper- β -glucan nanoconjugate against *S. rolfsii* causing tuberose stem rot”, the results obtained are presented below.

3.1 Morphological studies of *S. rolfsii*

The isolated pathogen produced cottony, creamy white to dull white mycelial growth on PDA. The mycelium showed a characteristic radial growth pattern with fan-shaped mycelial strands. Brown to dark brown, round to spherical sclerotia developed after 10-14 days, measuring 0.5-2.05

mm in diameter. Sclerotia were predominantly formed at the periphery of the colony, with a few scattered towards the centre. Microscopic examination of the mycelium revealed septate hyphae with clamp connections. Based on these cultural and morphological characteristics, the isolate was identified as *S. rolfii* (Table 1, Plate 1).

3.2 *in vitro* antifungal activity of chitosan nanoparticles against *S. rolfii*

Chitosan exhibited a concentration-dependent inhibitory effect on the mycelial growth of *S. rolfii*. The untreated control recorded the maximum mycelial diameter of 9.0 cm. With increasing concentration of chitosan, mycelial growth progressively decreased from 4.7 cm at 50 ppm to 0.2 cm at 700 ppm. The corresponding inhibition of mycelial growth increased from 48.15% at 50 ppm to 97.41% at 700 ppm.

The maximum inhibition was recorded at 700 ppm (97.41%), followed by 600 ppm (95.93%) and 500 ppm (92.59%). The growth pattern changed from fluffy growth at lower concentrations to compact and subsequently pin-point growth at higher concentrations, while the mycelial colour changed from cottony white to dull white (Table 2, Plate 2).

3.3 *in vitro* antifungal activity of β -glucan nanoparticles against *S. rolfii*

β -glucan exhibited a concentration-dependent inhibitory effect on the mycelial growth of *S. rolfii*. The untreated control recorded the maximum mycelial diameter of 9.0 cm. With increasing concentration of β -glucan, mycelial growth progressively decreased from 5.1 cm at 50 ppm to 0.3 cm at 700 ppm. The corresponding inhibition of mycelial growth increased from 43.33% at 50 ppm to 97.04% at 700 ppm.

The maximum inhibition was recorded at 700 ppm (97.04%), followed by 600 ppm (93.70%) and 500 ppm (90.37%). The growth pattern changed from fluffy growth at lower concentrations to compact and subsequently pin-point growth at higher concentrations, while the mycelial colour changed from cottony white to dull white (Table 3, Plate 3).

3.4 *in vitro* antifungal activity of chitosan-copper- β -glucan nanoconjugate against *S. rolfii*

The chitosan-copper- β -glucan nanoconjugate showed a pronounced concentration-dependent inhibition of *S. rolfii* mycelial growth. The untreated control recorded 9.0 cm mycelial growth, whereas the growth decreased progressively from 3.9 cm at 50 ppm to 0.2 cm at 300 ppm. The corresponding inhibition increased from 56.30% at 50 ppm to 98.15% at 300 ppm. The highest inhibition was recorded at 300 ppm (98.15%), followed by 250 ppm (94.81%) and 200 ppm (88.15%). At higher concentrations, the mycelial growth pattern changed from fluffy to compact and finally pinpoint, with the mycelial colour changing from cottony white to dull white (Table 4, Plate 4).

3.5 Effect of chitosan-copper- β -glucan nanoconjugate on sclerotial germination

The chitosan-copper- β -glucan nanoconjugate markedly reduced the germination of *S. rolfii* sclerotia in a concentration-dependent manner. Complete germination (100%) was recorded in the untreated control. Sclerotial germination decreased progressively with increasing nanoconjugate concentration, from 73.33% at 50 ppm to 0% at 300 ppm.

The lowest germination was recorded at 300 ppm, where complete inhibition of sclerotial germination was observed (Table 5, Plate 5).

4. Discussion

The present study demonstrated a clear concentration-dependent antifungal activity of chitosan nanoparticles, β -glucan nanoparticles and chitosan-copper- β -glucan nanoconjugate against *S. rolfii*. Chitosan nanoparticles reduced mycelial growth from 9.0 cm in the untreated control to 0.2 cm at 700 ppm, corresponding to 97.41% inhibition. The inhibition increased progressively from 48.15% at 50 ppm to 97.41% at 700 ppm. This concentration-dependent response agrees with Kheiri *et al.* [17], who reported significant inhibition of *Fusarium graminearum* by chitosan and chitosan nanoparticles, with maximum inhibition obtained at higher concentrations. Their study also observed dehydration, deformation and collapse of fungal hyphae following treatment with chitosan nanoparticles.

The antifungal activity of chitosan against *S. rolfii* is further supported by Eweis *et al.* [18], who evaluated chitosan and its thiourea derivative against several sugar-beet pathogens, including *S. rolfii*. They reported inhibitory effects on mycelial growth and on the germination of sclerotia, with *S. rolfii* sclerotia being particularly sensitive to the modified chitosan treatment. Chitosan-mediated disruption of fungal membrane integrity and cellular homeostasis is considered an important basis of this activity [10].

β -Glucan nanoparticles also exhibited a concentration-dependent inhibitory effect, reducing mycelial growth from 9.0 cm in the control to 0.3 cm at 700 ppm and producing 97.04% inhibition. Importantly, Chavanke and Dalvi [10] reported complete inhibition of sugarbeet sclerotium root rot causing *S. rolfii* at 160 ppm using β -glucan nanoparticles derived from *S. rolfii* and yeast. Their study specifically noted that *in vitro* investigations of β -glucan nanoparticles against *S. rolfii* were limited, making their finding directly relevant to the present investigation.

The chitosan-copper- β -glucan nanoconjugate exhibited the highest activity in the present study, reducing mycelial growth from 9.0 cm in the control to 0.2 cm at 300 ppm, corresponding to 98.15% inhibition. Thus, near-complete inhibition was achieved at less than half the concentration required for the individual chitosan nanoparticles and β -glucan nanoparticles, indicating that integration of copper within the chitosan- β -glucan nanosystem substantially enhanced antifungal potency. A plausible explanation is that the integrated nanostructure provides multiple antifungal interfaces and mechanisms. Chitosan can facilitate interaction with the fungal surface, whereas copper-containing nanostructures can perturb cellular integrity and redox homeostasis. Nanocomposite systems may additionally improve the effective presentation, dispersion or delivery of active components relative to their individual forms [15].

This interpretation is strongly supported by independent evidence. Rubina *et al.* [19] demonstrated that chitosan-copper nanocomposites exerted substantial fungicidal activity against the sclerotia-forming pathogens *S. rolfii* and *Rhizoctonia solani*. Their microscopic analyses showed loss of cytoplasmic contents, cytoplasmic coagulation, irregular hyphal morphology and destruction of fungal

hyphae following treatment, providing direct evidence that chitosan-copper nanocomposites can cause severe structural damage to fungal cells.

More recently, an independently reported chitosan-copper- β -glucan nanocomposite also demonstrated complete inhibition of *S. rolfsii* mycelial growth at 200 ppm, supporting the particularly strong antifungal potential of integrating chitosan, copper and β -glucan within a single nanosystem^[15]. The convergence of these findings with the present results strengthens the proposition that biopolymer-metal nanocomposites can provide an efficient platform for suppressing *S. rolfsii* at comparatively low concentrations.

The complete inhibition of *S. rolfsii* sclerotial germination by the chitosan-copper- β -glucan nanocomposite at 300 ppm is particularly important from a disease-management perspective. Unlike transient inhibition of vegetative mycelial growth, suppression of sclerotial germination directly targets the pathogen's principal survival and inoculum-producing phase. Sclerotia enable *S. rolfsii* to persist in soil and serve as a major source of primary inoculum; consequently, materials capable of simultaneously restricting mycelial development and preventing sclerotial germination may have greater

epidemiological value than treatments acting only on actively growing hyphae.

The present observation is consistent with Eweis *et al.*^[18], who reported pronounced inhibition of *S. rolfsii* sclerotial germination by chitosan-derived compounds and with Rubina *et al.*^[19], who demonstrated that chitosan-copper nanocomposites adversely affected both fungal hyphae and sclerotial development. Thus, the activity observed here is relevant not merely as an *in vitro* growth-inhibition phenomenon but as an indication that the nanocomposite may interfere with two biologically distinct stages of the *S. rolfsii* life cycle.

Overall, the findings establish a clear hierarchy of antifungal performance—chitosan nanoparticles and β -glucan nanoparticles showing strong but concentration-dependent inhibition, whereas the chitosan-copper- β -glucan nanoconjugate achieved near-complete inhibition at a considerably lower concentration and completely suppressed sclerotial germination. The results therefore identify the chitosan-copper- β -glucan nanoconjugate as the most promising candidate among the tested materials for subsequent greenhouse and field evaluation against *S. rolfsii*.

Table 1: Morphological characters of *S. rolfsii*

Sr. No.	Characters	Observations
1	Mycelium	Cottony mycelium
2	Mycelium shape	Radial growth with fan-shaped mycelial strands
3	Mycelium colour	Creamy white to dull white
4	Sclerotial colour	Brown to dark brown
5	Sclerotial shape	Round to spherical
6	Sclerotial size	0.5-2.05 mm in diameter
7	Days required for sclerotia formation	10-14 days
8	Sclerotial distribution	Predominantly at colony periphery, with few scattered toward the centre

*Observations based on cultural and morphological examination

Table 2: *In vitro* antifungal activity of chitosan against *S. rolfsii*

Tr. No.	Concentration (ppm)	Mean mycelial diameter* (cm)	Percent growth inhibition	Growth pattern	Mycelial colour
T ₁	Control	9.0	0.00	Fluffy	Cottony white
T ₂	50	4.7	48.15	Fluffy	Cottony white
T ₃	100	3.8	57.78	Compact	Cottony white
T ₄	200	3.2	64.44	Compact	Cottony white
T ₅	300	1.8	80.37	Compact	Dull white
T ₆	400	1.2	87.04	Compact	Dull white
T ₇	500	0.7	92.59	Compact	Dull white
T ₈	600	0.4	95.93	Pinpoint	Dull white
T ₉	700	0.2	97.41	Pinpoint	Dull white
SE(m)±	0.09				
C.D. (0.01)	0.37				

*Mean of three replications

Table 3: *In vitro* antifungal activity of β -glucan against *S. rolfsii*

Tr. No.	Concentration (ppm)	Mean mycelial diameter* (cm)	Percent growth inhibition	Growth pattern	Mycelial colour
T ₁	Control	9.0	0.00	Fluffy	Cottony white
T ₂	50	5.1	43.33	Fluffy	Cottony white
T ₃	100	4.0	55.56	Fluffy	Cottony white
T ₄	200	3.4	61.85	Fluffy	Dull white
T ₅	300	2.2	75.93	Compact	Dull white
T ₆	400	1.5	83.70	Compact	Dull white
T ₇	500	0.9	90.37	Compact	Dull white
T ₈	600	0.6	93.70	Pinpoint	Dull white
T ₉	700	0.3	97.04	Pinpoint	Dull white
SE(m)±	0.09				
C.D. (0.01)	0.38				

*Mean of three replications

Table 4: *In vitro* antifungal activity of chitosan-copper- β -glucan nanoconjugate against *S. rolfsii*

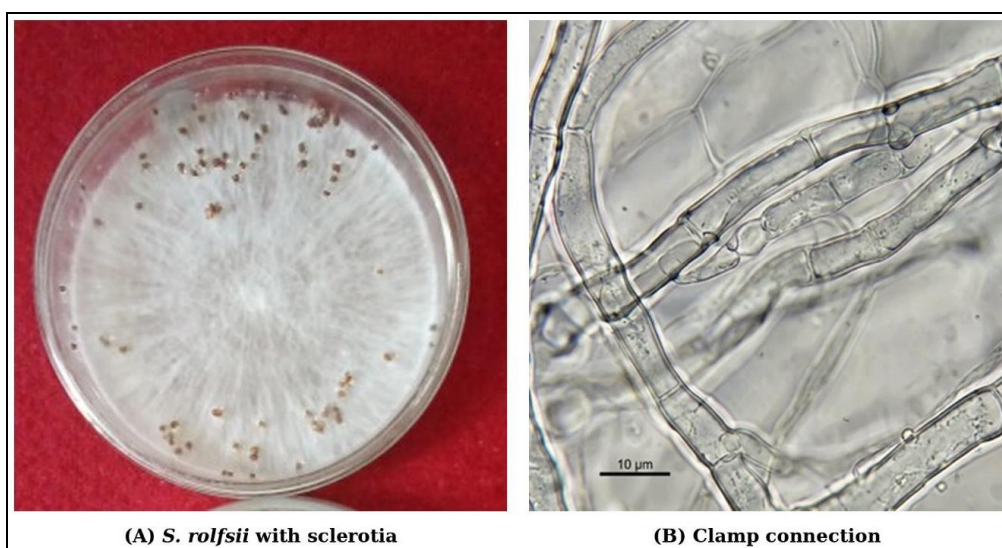
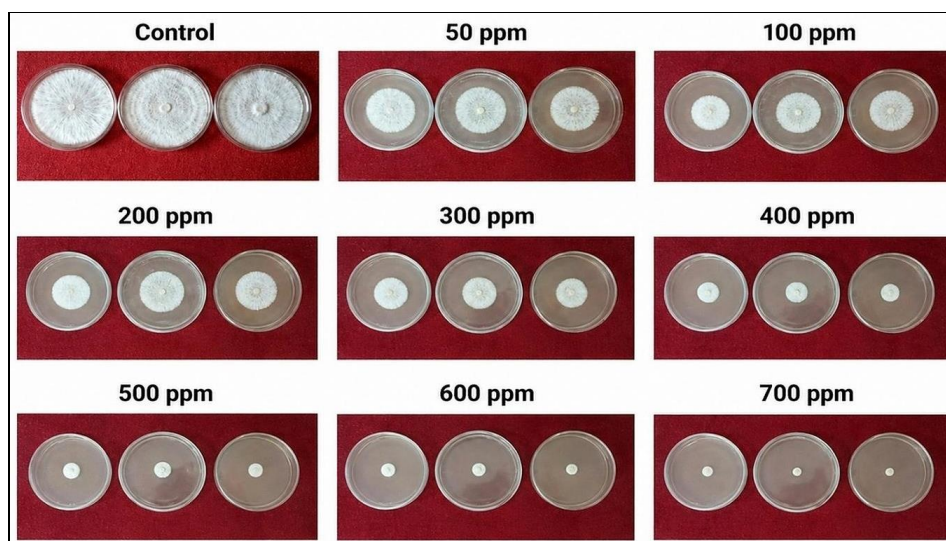
Tr. No.	Concentration (ppm)	Mean mycelial diameter* (cm)	Percent growth inhibition	Growth pattern	Mycelial colour
T ₁	Control	9.0	0.00	Fluffy	Cottony white
T ₂	50	3.9	56.30	Fluffy	Cottony white
T ₃	100	2.3	74.81	Compact	Dull white
T ₄	150	1.6	81.85	Compact	Dull white
T ₅	200	1.1	88.15	Compact	Dull white
T ₆	250	0.5	94.81	Pinpoint	Dull white
T ₇	300	0.2	98.15	Pinpoint	Dull white
SE(m)±	0.07				
C.D. (0.01)	0.30				

*Mean of three replications

Table 5: Effect of chitosan-copper- β -glucan nanoconjugate on sclerotial germination of *S. rolfsii*

Tr. No.	Concentration (ppm)	Mean Percent sclerotial germination
T ₁	Control	100.00
T ₂	50	73.33
T ₃	100	53.33
T ₄	150	33.33
T ₅	200	26.67
T ₆	250	13.33
T ₇	300	0.00
SE(m)±	0.28	
C.D. (0.01)	1.15	

*Mean of three replications

**Plate 1:** Morphology of *S. rolfsii* — (A) *S. rolfsii* with sclerotia; (B) Clamp connection**Plate 2:** *In vitro* antifungal activity of chitosan nanoparticles against *S. rolfsii*

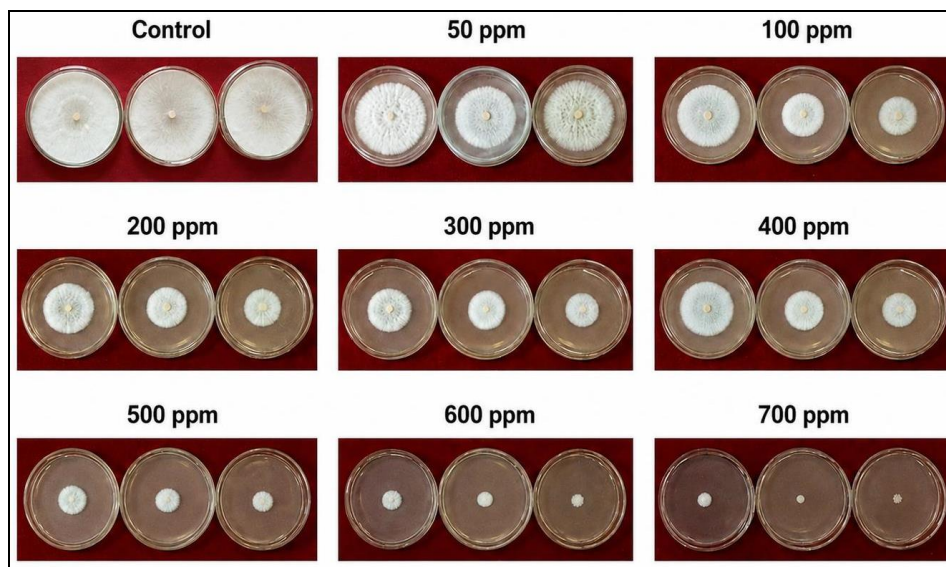


Plate 3: *In vitro* antifungal activity of β -glucan nanoparticles against *S. rolfsii*

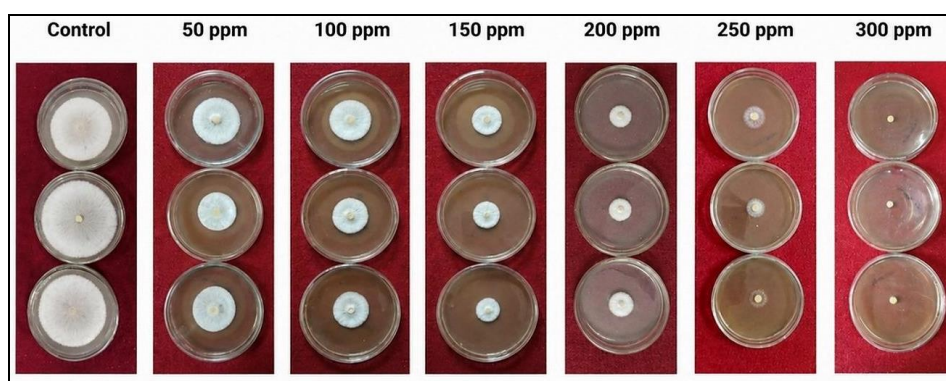


Plate 4: *In vitro* antifungal activity of chitosan-copper- β -glucan nanoconjugate against *S. rolfsii*

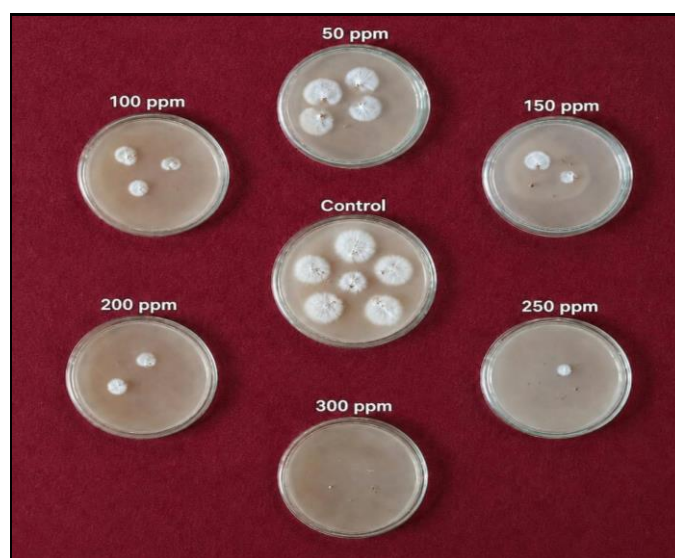


Plate 5: Effect of chitosan-copper- β -glucan nanoconjugate on sclerotial germination of *S. rolfsii*

5. Conclusion

The present study demonstrated that chitosan nanoparticles, β -glucan nanoparticles and chitosan-copper- β -glucan nanoconjugate exhibited significant, concentration-dependent antifungal activity against *S. rolfsii* under *in vitro* conditions. Chitosan and β -glucan nanoparticles recorded maximum mycelial growth inhibition of 97.41 and 97.04%, respectively, at 700 ppm. The chitosan-copper- β -glucan nanoconjugate showed the highest inhibition of 98.15% at

300 ppm and completely inhibited sclerotial germination at the same concentration. This substantially lower effective concentration, together with inhibition of both vegetative growth and the pathogen's persistent survival structure, identifies chitosan-copper- β -glucan nanoconjugate as the most promising antifungal material evaluated. The findings are consistent with previous evidence that chitosan-copper nanocomposites can strongly suppress *S. rolfsii* growth and sclerotial development.

Overall, the results provide compelling *in vitro* evidence for the potential of chitosan-copper- β -glucan nanoconjugate as a nano-enabled strategy for *S. rolfsii* management and provide a strong basis for its further validation under greenhouse and field conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Bharathi TU, Kumar R, Nair SA, Umamaheswari R, Sonavane P, Kalaivanan D, *et al.* Evaluation of tuberose genotype IIHR 17-23SP-08 (IC0642158) for flower yield, quality and response to biotic stress. *Journal of Horticultural Sciences*. 2023;18(1):60-66.
2. Salomé-Abarca LF, Márquez-López RE, López MG. *Agave amica* a potential model for the study of agavins metabolism. *Scientific Reports*. 2023;13:19888.
3. Boopathi P, Jayasekhar M, Rajinimala N, Gomathy M, Prem Joshua J. Studies on varietal reaction of tuberose against stem rot diseases caused by *S. rolfsii*. *International Journal of Current Microbiology and Applied Sciences*. 2020;9(7):665-671.
4. Vimal S, Deivamani M, Sudha A, Vennila MA, Indhumathi K, Senthilkumar P. Studies on morphological and molecular traits of tuberose diseases and its chemical and biological control. *Journal of Advances in Biology & Biotechnology*. 2024;27(9):155-170.
5. Punja ZK. The biology, ecology and control of *S. rolfsii*. *Annual Review of Phytopathology*. 1985;23:97-127.
6. Kator L, Hosea ZY, Oche OD. *S. rolfsii*; causative organism of southern blight, stem rot, white mold and sclerotia rot disease. *Annual Research & Review in Biology*. 2015;6:78-89.
7. Dwivedi SK, Prasad G. Integrated management of *S. rolfsii*: An overview. *European Journal of Biomedical and Pharmaceutical Sciences*. 2016;3(11):137-146.
8. Chowdary GBSM, Jameema G, Charishma KV. Collar and stem rot pathogen—*S. rolfsii*: A review. *Plant Archives*. 2024;24(1):67-72.
9. Malerba M, Cerana R. Chitosan effects on plant systems. *International Journal of Molecular Sciences*. 2016;17(7):996.
10. Chavanke SN, Dalvi SG. Bioinspired nano-architected chitosan- β -glucan nanocomposite as an elicitor for disease management sustainably. *Scientific Reports*. 2025;15:34031.
11. Tran CD, Makuvaza J, Munson E, Bennett B. Biocompatible copper oxide nanoparticle composites from cellulose and chitosan: Facile synthesis, unique structure and antimicrobial activity. *ACS Applied Materials & Interfaces*. 2017;9(49):42503-42515.
12. Ashrafi M, Bayat M, Mortazavi P, Hashemi SJ, Meimandipour A. Antimicrobial effect of chitosan-silver-copper nanocomposite on *Candida albicans*. *Journal of Nanostructure in Chemistry*. 2020;10:87-95.
13. de Souza MB, Stamford NP, Silva EVN, Berger LR, Silva SCER, Costa AF, *et al.* Defense response by inter-active bio-protector and chitosan to *S. rolfsii* wilt disease on cowpea, Brazilian Oxisol. *African Journal of Agricultural Research*. 2018;13(21):1053-1062.
14. Shoaib A, Iqbal N, Fatima Q, Malik B. Quantitative analysis of chitosan concentration-dependent modulation of phytopathogenic fungal growth: Chitosan concentration-dependent modulation of phytopathogenic fungi. *Plantarum*. 2024;6(1):130-137.
15. Chavanke SN, Dalvi SG. Biopolymer-engineered novel chitosan-copper- β -glucan nanocomposite: A sustainable antifungal innovation for enhanced plant immunity and crop yield. *International Journal of Biological Macromolecules*. 2026;366:152472.
16. Panse VG, Sukhatme PV. *Statistical Methods for Agricultural Workers*. 4th ed. New Delhi: Indian Council of Agricultural Research; 1985. 359 p.
17. Kheiri A, Moosawi Jorf SA, Malihipour A, Saremi H, Nikkhah M. Application of chitosan and chitosan nanoparticles for the control of Fusarium head blight of wheat (*Fusarium graminearum*) *in vitro* and greenhouse. *International Journal of Biological Macromolecules*. 2016;93(Pt A):1261-1272.
18. Eweis M, Elkholy SS, Elsabee MZ. Antifungal efficacy of chitosan and its thiourea derivatives upon the growth of some sugar-beet pathogens. *International Journal of Biological Macromolecules*. 2006;38(1):1-8.
19. Rubina MS, Vasil'kov AY, Naumkin AV, Shtykova EV, Abramchuk SS, Alghuthaymi MA, *et al.* Synthesis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. *Journal of Nanostructure in Chemistry*. 2017;7(3):249-258.
20. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 1947;159:850.