

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
Indexing: Embase
IJABR 2026; SP 10(10): 945-953
www.biochemjournal.com
Received: 10-08-2026
Accepted: 29-09-2026
Published: 10-10-2026

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

Chavanke SN

Scientific Officer, 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

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

Chitosan–copper– β -glucan nanoconjugate against *Sclerotium Rolfsii* causing stem rot in tuberose: Disease incidence and defence-related responses

Mahima J¹, Lohate SR², Dalvi SG³, Chavanke SN⁴, Karade VM⁵, Lubna Ghajal⁶

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i10SI.10405>

ABSTRACT

Stem rot caused by *Sclerotium rolfsii* is a serious soil-borne disease of tuberose, and approaches that suppress the disease while activating host defence are needed. A chitosan–copper– β -glucan nanoconjugate was evaluated under pot-culture conditions for management of stem rot and for selected defence-related biochemical responses. Bulb dipping at planting was followed by foliar sprays at 30, 45 and 60 days after planting with chitosan nanoparticles and β -glucan nanoparticles (50 ppm each) or the nanoconjugate (50, 75, 100 and 125 ppm). Copper oxychloride and microbial antagonists were included for comparison. Disease incidence was 91.67% in the untreated control and fell to 25.00, 16.67, 0.00 and 0.00% with the nanoconjugate at 50, 75, 100 and 125 ppm, respectively (72.22, 80.56, 100 and 100% disease control). The highest chitinase (1.56 U mg⁻¹ protein), β -1,3-glucanase (0.66 U mg⁻¹ protein) and total phenol (5.91 mg g⁻¹ fresh weight) values were recorded at 100 ppm. Polyphenol oxidase activity was highest at 75 and 100 ppm (0.24 Δ absorbance min⁻¹ g⁻¹ fresh weight). Total chlorophyll increased by 21.74% at 125 ppm. The 100 and 125 ppm treatments were statistically at par. The nanoconjugate, particularly at 100 ppm, markedly suppressed stem rot and enhanced hydrolytic enzymes, phenolic defence and photosynthetic pigments in tuberose. These findings identify the chitosan–copper– β -glucan nanoconjugate as a promising candidate for further greenhouse and field validation for sustainable management of *Sclerotium rolfsii* in tuberose.

Keywords: B-1,3-glucanase, Chitinase, Chitosan–copper– β -glucan nanoconjugate, *Sclerotium rolfsii*, Stem rot, Tuberose

1. Introduction

Tuberose (*Polianthes tuberosa*) is an important bulbous ornamental crop cultivated for its fragrant flower spikes and value in floriculture. Stem rot caused by *Sclerotium rolfsii* is an important soil-borne disease of tuberose. The pathogen produces persistent sclerotia and can cause basal stem and root infection, wilting, plant collapse and characteristic white cottony mycelium with brown sclerotia (Boopathi, 2023; Wheeler, 1969) [1, 2]. Management of *S. rolfsii* is difficult because of its soil-borne nature and survival through sclerotia. Studies in tuberose have demonstrated the potential of biological control agents such as *Trichoderma viride* and *Pseudomonas fluorescens*, but additional sustainable disease-management approaches are needed (Sankara Reddy et al., 2021) [3, 4].

Chitosan is a biodegradable biopolymer with antimicrobial and plant defence-eliciting properties. Chitosan nanoparticles and chitosan-based nanocomposites have been investigated as nano-enabled tools for phytopathogen management (Chandra et al., 2015; Saberi Riseh et al., 2024) [5, 6]. β -Glucans can function as elicitor molecules and stimulate plant defence responses, including changes in defence-associated enzymes such as chitinase, β -1,3-glucanase and polyphenol oxidase (PPO) (Saberi Riseh et al., 2023) [7]. Chitin- and chitosan-derived elicitors have likewise been associated with activation of defence-related enzymes (Saberi Riseh et al., 2024) [8].

Recent advances in biopolymer-based nanotechnology have shown the potential of combining chitosan and β -glucan with copper in multifunctional nanocomposites. Chitosan and β -glucan are antimicrobial and can act as pathogen-associated molecular patterns recognised by plant pattern-recognition receptors, thereby activating defence signalling, while copper nanoparticles in the matrix may add antifungal activity (Chavanke et al., 2026; Chavanke et al., 2025; Chavanke et al., 2022; Vanti et al., 2020) [9, 10, 11, 12]. Chitosan– β -glucan nanocomposites have accordingly been investigated as elicitors against fungal pathogens (Chavanke et al., 2025) [10], and β -glucan-mediated responses are associated with induction of pathogenesis-related proteins such as β -1,3-glucanase and chitinase and of enzymes of phenolic metabolism (Chavanke et al., 2022) [11].

Chitosan–copper–β-glucan (CN–Cu–β-glucan) nanocomposites showed strong antifungal activity against *S. rolfii* and enhanced chitinase and β-glucanase activities in another crop system (Chavanke *et al.*, 2026) [9]. However, their application against stem rot of tuberose, their association with defence-related biochemical responses and the contribution of individual components remain insufficiently documented.

Therefore, the present study was undertaken to evaluate the efficacy of the CN–Cu–β-glucan nanoconjugate for suppression of stem rot disease caused by *S. rolfii* and to assess its association with defence-related responses.

2. Materials and Methods

2.1 Experimental site

The investigation was carried out during 2025–2026. The pot culture experiment was conducted at AICRP on Floriculture, Zonal Agricultural Research Station (ZARS), Ganeshkhind, Pune, and biochemical analyses were carried out at Vasantdada Sugar Institute (VSI), Manjari Budruk, Pune.

2.2 Plant material and pathogen

Tuberose (*Polianthes tuberosa* L.) cultivar Phule Rajani was used as the test plant, and *Sclerotium rolfii*-infested sick soil was used to establish disease pressure. The sick soil was thoroughly mixed before

filling the experimental pots, and healthy tuberose bulbs were planted in the infested soil under conditions conducive to disease development. The CN–Cu–β-glucan nanoconjugate used in the present study was obtained from Vasantdada Sugar Institute (VSI), Manjari, Pune, and was prepared and characterized following the procedure described in references (Chavanke *et al.*, 2026; Chavanke *et al.*, 2025) [9, 10]. The nanoconjugate was subsequently used at the concentrations specified for the respective treatments.

2.3 Pot culture treatments

The pot culture experiment comprised eleven treatments, including an untreated control (T_{11}), with three replications per treatment. Copper oxychloride (0.2%) was applied as soil drenching at 40 and 50 days after planting (DAP) (T_1). Chitosan nanoparticles (50 ppm) and β-glucan nanoparticles (50 ppm) were applied through bulb dipping at planting followed by foliar sprays at 30, 45 and 60 DAP (T_2 and T_3 , respectively). The CN–Cu–β-glucan nanoconjugate was evaluated at 50, 75, 100 and 125 ppm (T_4 – T_7), with bulb dipping at planting followed by foliar application at 30, 45 and 60 DAP. *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were applied through bulb dipping at planting followed by soil drenching at 40 DAP (T_8 – T_{10} , respectively). The untreated control (T_{11}) received no treatment. The complete treatment schedule is presented in Table 1.

2.4 Disease incidence and disease control

Disease incidence was calculated as follows (Wheeler, 1969) [2]:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Disease control was calculated as:

$$\text{Disease control (\%)} = \frac{\text{Disease incidence in control} - \text{Disease incidence in treatment}}{\text{Disease incidence in control}} \times 100$$

Disease control was calculated separately for each replication and averaged to obtain the treatment-wise disease control percentage presented in Table 3.

2.5 Leaf sampling and biochemical analysis

Following the third foliar spray, leaf samples were collected after 72 h and immediately preserved in liquid nitrogen until biochemical analysis. For biochemical analysis, 200 mg of frozen leaf tissue was accurately weighed, powdered under chilled conditions, and used for preparation of the respective extracts.

Chitinase activity was estimated by the 3,5-dinitrosalicylic acid (DNSA) colorimetric method using chitin as the substrate and *N*-acetyl-D-glucosamine (NAG) as the standard. A standard curve was prepared using known concentrations of NAG, and the amount of NAG released during the enzymatic reaction was calculated from the corresponding standard curve. Absorbance was recorded at 540 nm, and chitinase activity was expressed as U mg^{−1} protein.

β-1,3-Glucanase activity was determined using laminarin as the substrate and glucose as the standard. A glucose standard curve was prepared using known concentrations of glucose, and the amount of glucose released during the enzymatic reaction was calculated from the corresponding standard curve. Absorbance was recorded at 540 nm, and β-1,3-glucanase activity was expressed as U mg^{−1} protein.

Total soluble protein content was determined by the Bradford assay and used to normalize the activities of chitinase and β-1,3-glucanase. Total phenol content was estimated by the Folin–Ciocalteu method using catechol as the standard. A catechol standard curve was prepared using known concentrations of catechol, and the phenol content was calculated from the corresponding standard curve. Absorbance was recorded at 650 nm, and total phenol content was expressed as mg g^{−1} fresh weight (FW).

Polyphenol oxidase (PPO) activity was assayed using catechol as the substrate. The enzymatic reaction was monitored spectrophotometrically at 420 nm, and PPO activity was calculated from the change in

absorbance per unit time. The activity was expressed as Δ absorbance min^{−1} g^{−1} FW. Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents were estimated following the method of Arnon (1949).

The biochemical parameters were determined to assess treatment-associated changes in selected defence-related responses of tuberose plants following application of the CN–Cu–β-glucan nanoconjugate and other treatments.

2.6 Statistical analysis

Data were analysed using a completely randomized design. Suitable transformation was applied where necessary and critical difference (CD) at the 5% level was calculated where the *F*-test was significant (Gomez *et al.*, 1984; Panse *et al.*, 1985) [13, 14]. The heatmap was used to visualize the relative variation and clustering patterns of the measured biochemical and physiological parameters across treatments.

3. Results

3.1 Effect of treatments on defence-related hydrolytic enzymes

The treatments markedly influenced β-glucanase and chitinase activities compared with the untreated control (T_{11}) (Table 2). β-Glucanase activity in T_{11} was 0.43 U mg^{−1} protein. CN–Cu–β-glucan at 100 ppm (T_6) recorded the highest activity (0.66 U mg^{−1} protein; 53.49% increase over T_{11}), followed by 125 ppm (T_7 ; 0.62; 44.19%), 75 ppm (T_5 ; 0.56; 30.23%) and 50 ppm (T_4 ; 0.50; 16.28%). T_6 and T_7 were statistically at par with each other and significantly higher than T_{11} , whereas all other treatments remained at par with the control despite numerical differences.

Chitinase activity followed a similar trend. The untreated control exhibited 0.88 U mg^{−1} protein, whereas T_6 showed the highest activity (1.56 U mg^{−1} protein; 77.27% increase), followed by T_7 (1.45;

64.77%) and T_5 (1.29; 46.59%). T_5 , T_6 and T_7 were significantly superior to the control, whereas T_4 and T_9 (27.27 and 30.68% increases) remained at par with T_{11} . T_6 and T_7 were statistically at par for both hydrolytic enzymes.

3.2 Effect of different treatments on phenolic accumulation and polyphenol oxidase activity

Total phenol content in T_{11} was 5.51 mg g⁻¹ FW (Table 2). T_6 recorded the highest value (5.91 mg g⁻¹ FW; 7.26% increase), closely followed by T_7 (5.90; 7.08%) and T_2 (5.83; 5.81%). All treatments except copper oxychloride (T_1 ; 2.36% increase), which was at par with T_{11} , were significantly higher than the untreated control. PPO activity showed a much stronger response than total phenols. T_{11} recorded 0.09 Δ absorbance min⁻¹ g⁻¹ FW, whereas T_5 and T_6 showed the highest activity (0.24; 166.67% increase), followed by T_7 and T_8 (0.20; 122.22%) and T_3 (0.19; 111.11%). T_9 , T_{10} and T_1 remained at par with T_{11} . The largest statistically supported enhancement of PPO was thus associated with the nanoconjugate, particularly T_5 and T_6 .

3.3 Effect of different treatments on photosynthetic pigments

The nanoconjugate also modified pigment accumulation (Tables 2 and 3). Chlorophyll *a* (Chl *a*) was 0.14 mg g⁻¹ FW in T_{11} , and T_5 , T_6 and T_7 each recorded 0.16 mg g⁻¹ FW (14.29% increase), forming a significantly higher group; the other treatments did not differ significantly from the control. For chlorophyll *b* (Chl *b*), T_4 – T_7 recorded 0.11 mg g⁻¹ FW against 0.10 in T_{11} (10.0% increase) and were assigned to higher statistical groups. The displayed mean of T_3 equalled that of T_{11} , although its statistical grouping differed, owing to rounding of the displayed means.

The effect was more evident for total chlorophyll. T_7 recorded the highest concentration (0.28 mg g⁻¹ FW; 21.74% increase over T_{11}), followed by T_5 and T_6 (0.27; 17.39%); these three were significantly higher than the control, whereas T_3 , T_4 and the remaining treatments were at par with T_{11} .

Carotenoid concentration was 0.01 mg g⁻¹ FW across all treatments after rounding, so no meaningful percentage change can be inferred, and the Chl *a*/*b* ratio did not differ significantly among treatments.

3.4 Disease incidence and disease suppression

Disease development was strongly influenced by the treatments (Table 3). The untreated control (T_{11}) recorded 91.67% disease incidence. The CN–Cu– β -glucan nanoconjugate at 100 ppm (T_6) and

125 ppm (T_7) completely suppressed visible disease incidence, with 0.00% incidence and 100.00% disease control in both treatments. These two concentrations were statistically at par with each other and occupied the highest statistical group for disease suppression.

The 75 ppm nanoconjugate (T_5) reduced disease incidence to 16.67% (80.56% disease control) and was statistically at par with T_6 and T_7 . T_4 (50 ppm) recorded 25.00% incidence (72.22% control), T_2 and T_3 each 33.33% (63.89%), and copper oxychloride (T_1) 50.00% (44.44%). Among the microbial treatments, *Trichoderma viride* (T_8), *Pseudomonas fluorescens* (T_9) and *Bacillus subtilis* (T_{10}) recorded 58.33, 66.67 and 66.67% incidence (36.11, 27.78 and 25.00% control). Overall, the nanoconjugate produced the strongest integrated response across defence enzymes, phenolic metabolism, pigments and disease suppression, with no statistically detectable additional benefit at 125 ppm over 100 ppm.

3.5 Multivariate clustering and heatmap analysis of treatment responses

Standardized heatmap analysis revealed distinct treatment-dependent patterns across disease, pigment, phenolic and enzyme variables (Fig 1). Hierarchical clustering separated the nanoconjugate treatments (T_4 – T_7) from the untreated and conventional/biological treatments, with T_6 and T_7 showing the most closely related profiles. The T_4 – T_7 cluster showed positive standardized responses for PPO, chlorophylls, β -glucanase and chitinase and a strongly negative pattern for disease incidence; T_5 was broadly similar but weaker for some variables, and T_4 intermediate. T_{11} was characterized by high disease incidence and comparatively low phenolics, β -glucanase, chitinase and chlorophyll, and T_1 and T_3 partly resembled it, whereas T_8 , T_{10} , T_2 and T_9 grouped separately with heterogeneous profiles and no consistent combination of enhanced defence and disease control.

At the variable level, Chl *a*, Chl *b* and total chlorophyll clustered together, as did β -glucanase and chitinase, whereas disease incidence was inversely related to disease control and separated from the defence and photosynthetic variables; PPO and total phenols occupied an intermediate position. Overall, the clustering confirmed the individual analyses, identifying T_4 – T_7 as the most distinct response group, characterized by low disease incidence, high disease control, enhanced hydrolytic enzymes and PPO, and improved chlorophyll status.

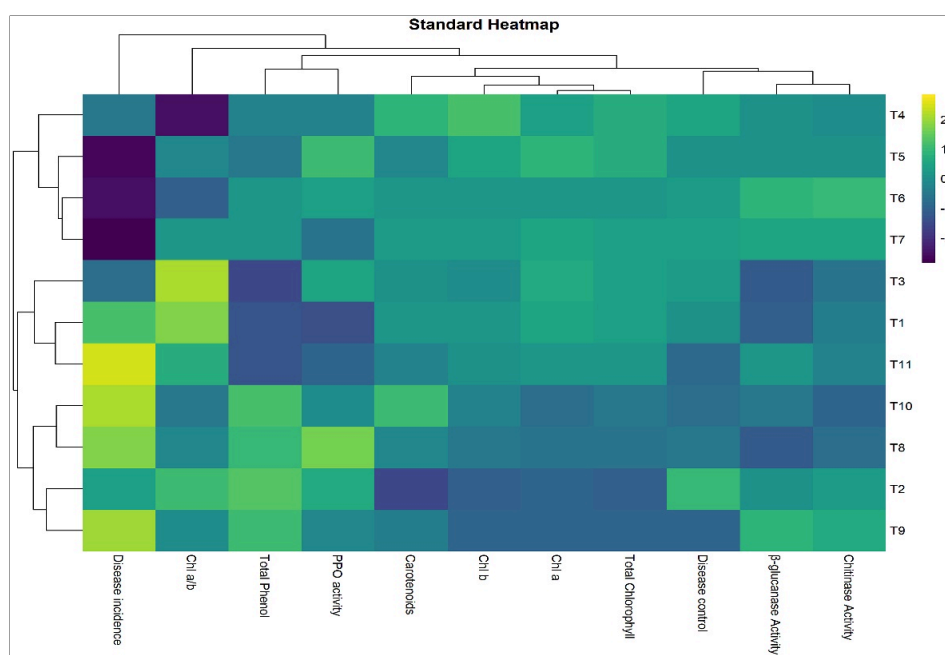


Figure 1. Hierarchical clustering heatmap of standardized treatment responses in tuberose.

4. Discussion

4.1 CN–Cu– β -glucan nanoconjugate enhances coordinated enzymatic defence

The present study demonstrated that the CN–Cu– β -glucan nanoconjugate elicited a coordinated biochemical defence response in tuberose, particularly at 75–125 ppm. The strongest response was observed for β -glucanase and chitinase, with increases of up to 53.49 and 77.27%, respectively, over the untreated control. Notably, the 100 ppm treatment (T_6) produced the highest numerical activities, while 125 ppm (T_7) was statistically at par for the principal hydrolytic enzymes, indicating that the response approached a concentration-dependent plateau rather than increasing further at the highest concentration tested. The simultaneous enhancement of β -glucanase and chitinase is particularly relevant to fungal disease resistance because these enzymes target structurally important polysaccharides of fungal cell walls. Plant β -1,3-glucanases and chitinases are widely recognized as pathogenesis-related hydrolytic enzymes whose induction can contribute to degradation of invading fungal structures and restriction of pathogen development. This interpretation is supported by (Chun and Chandrasekaran) [15], who demonstrated that chitosan nanoparticles enhanced β -1,3-glucanase and chitinase gene expression in tomato and were associated with increased resistance to *Fusarium andiyazi*. Thus, the elevated enzyme activities observed in the present tuberose plants provide a plausible biochemical basis for the markedly reduced disease development under CN–Cu– β -glucan treatment. The chitosan component is also consistent with the recognized elicitor activity of chitosan-derived materials. (Chandra *et al.*) [5] showed that chitosan nanoparticles can act as positive modulators of innate immune responses in plants and stimulate multiple defence-associated responses following application. More specifically, (Brulé *et al.*) [16] provided evidence that chitin/chitosan-derived oligomers can function as pathogen-associated molecular patterns in grapevine and demonstrated involvement of LysM receptor kinases in chitoooligosaccharide-triggered immune signalling. Although the corresponding receptor-mediated mechanism was not examined in tuberose, these findings provide a credible mechanistic framework in which the chitosan fraction of the nanoconjugate can act as a defence-associated stimulus. The β -glucan component may provide an additional elicitor signal. β -Glucan perception is highly dependent on polymer structure and molecular organization. (Shinya *et al.*) [17] isolated a β -1,3-,1,6-oligoglucan elicitor from *Alternaria alternata* that strongly induced chitinase activity in tobacco cells and showed that specific structural features of the glucan were important for elicitor activity. Likewise, (Aziz *et al.*) [18] demonstrated that laminarin, a β -1,3-glucan elicitor, stimulated defence responses in grapevine and reduced development of *Botrytis cinerea* and *Plasmopara viticola*. These studies support the biological plausibility of β -glucan-mediated stimulation of defence metabolism in the present experiment.

Importantly, T_4 (50 ppm CN–Cu– β -glucan) showed numerically lower disease incidence and generally higher hydrolytic-enzyme activity than the individual chitosan- and β -glucan-nanoparticle treatments (T_2 and T_3), although these differences were not statistically significant at the 5% level (Tables 2 and 3). The response therefore suggests, but does not establish, that combining the two polysaccharide components within the nanoconjugate generated a broader or more effective treatment response than either component alone.

4.2 Activation of phenolic metabolism and PPO strengthens the defence phenotype

Total phenol content increased by approximately 7% at T_6 and T_7 , whereas PPO activity increased by approximately 167% at T_5 and T_6 . The much larger PPO response suggests that the treatment stimulated phenolic turnover and oxidation rather than simple accumulation of the soluble phenolic pool.

PPO is a copper-containing metalloenzyme and has been extensively implicated in plant defence against pathogens and herbivores. (Zhang and Sun) [19] summarized evidence showing that PPO induction is as-

sociated with plant defence and that PPO-mediated oxidation of phenolic substrates can contribute to biochemical barriers against biotic stress. In this context, the pronounced increase in PPO activity observed under T_5 and T_6 , together with the moderate increase in total phenolics, indicates enhanced activation of the phenolic defence machinery.

The presence of copper in the nanoconjugate is particularly relevant because Cu is an essential micronutrient involved in photosynthesis, respiration, antioxidant metabolism and the activity of several plant enzymes. (Wang *et al.*) [20] emphasized that Cu participates in multiple physiological and biochemical processes, while also noting that excessive Cu can generate reactive oxygen species and cause phytotoxicity. The strong PPO response without a corresponding decline in chlorophyll in the present study suggests that the tested CN–Cu– β -glucan concentrations were physiologically tolerated and may have supplied Cu within a biologically effective range.

The association between chitosan nanoparticles and increased phenolic metabolism has also been reported experimentally. (Chandra *et al.*) [5] documented enhanced defence-related responses following chitosan nanoparticle treatment, while (Mawale and Giridhar) [21] reported biochemical and growth responses following chitosan nanoparticle application in *Capsicum* under greenhouse conditions. These observations are consistent with the present increase in phenolics and PPO and suggest that the nanoconjugate response extends beyond hydrolytic defence to include secondary metabolism.

4.3 Maintenance of photosynthetic pigments accompanies enhanced disease protection

Chl *a* increased by 14.29% under T_5 – T_7 , Chl *b* by 10% under T_4 – T_7 and total chlorophyll by 17.39–21.74% under T_5 – T_7 , with T_7 highest. The unchanged Chl *a/b* ratio indicates that the treatment increased total pigment abundance rather than altering the relative chlorophyll fractions. Maintenance of chlorophyll in the strongly protected plants is consistent with reduced physiological disruption under disease pressure.

Chitosan nanoparticles can influence plant physiology as well as disease expression (Mawale *et al.*, 2024) [21], and chitosan nanoparticle-mediated protection has been linked to defence signalling (Sarkar *et al.*, 2022; Sathiyabama *et al.*, 2016) [22, 23]; for example, (Sarkar *et al.*) [22] showed that chitosan nanoparticles reduced *Alternaria* leaf spot in chilli through a nitric oxide-dependent response. Nitric oxide was not measured in the present study.

Copper nutrition may further contribute to the pigment response, since Cu is essential for photosynthetic electron transport although excess Cu disrupts chloroplast function (Wang *et al.*, 2024) [20]. The absence of pigment deterioration indicates no obvious phytotoxicity at the tested concentrations.

4.4 Strong disease suppression is associated with a biochemical response plateau at 100–125 ppm

The disease response provides the clearest phenotypic evidence of treatment efficacy. Disease incidence declined from 91.67% in T_{11} to 16.67% with T_5 and to 0% with T_6 and T_7 , corresponding to the reported disease-control values of 80.56 and 100%, respectively. Importantly, T_6 and T_7 were statistically at par, indicating that increasing the concentration from 100 to 125 ppm did not provide a statistically demonstrable additional reduction in disease incidence.

This response pattern corresponded closely with the biochemical data. T_6 showed the highest β -glucanase and chitinase activities, while T_7 remained statistically comparable; T_5 and T_6 generated the highest PPO response; and T_7 produced the highest total chlorophyll concentration. Therefore, the most effective disease-suppression range also coincided with strong activation of multiple defence-associated traits. Rather than indicating a simple linear dose response, the findings suggest that the nanoconjugate reaches an effective physiological response threshold within the 75–125 ppm range. Such concentration dependence is compatible with established responses to chitosan nanoparticles. (Chandra *et al.*) [5] demonstrated that nanoparticulate

chitosan can enhance innate immune responses, while (Sarkar *et al.*) [22] reported concentration-dependent antifungal activity and disease protection associated with chitosan nanoparticles. Thus, the plateau between 100 and 125 ppm in tuberose may represent saturation of the host response, rather than evidence that the higher concentration is intrinsically less effective. The comparison among the nanoconjugate concentrations is especially informative. At 50 ppm, T_4 reduced disease incidence to 25%, whereas increasing the concentration to 75 ppm reduced it further to 16.67%, and 100–125 ppm eliminated detectable disease in the treated plants. This progressive response, together with the parallel increase in hydrolytic enzyme activity, supports a biologically coherent relationship between treatment intensity, host defence activation and disease restriction.

The performance of the combined formulation relative to the single-component nanoparticle treatments also deserves emphasis. Chitosan nanoparticles and β -glucan nanoparticles alone each produced intermediate disease control (63.89%), whereas the combined formulation at 100 and 125 ppm (T_6 and T_7) produced significantly greater suppression; at 50 and 75 ppm (T_4 and T_5) the advantage over the single-component treatments was numerical only. Previous studies have independently established biological activity for both chitosan-derived elicitors and β -glucan-derived elicitors: chitosan/chitooligosaccharides can stimulate immune signalling through LysM-associated perception mechanisms (Brulé *et al.*, 2019) [16], whereas β -glucan elicitors can activate defence responses and increase disease resistance (Shinya *et al.*, 2006; Aziz *et al.*, 2003) [17, 18]. The present results therefore suggest that incorporation of these components into a CN–Cu– β -glucan formulation can generate a broad defence-associated phenotype.

4.5 An integrated model for CN–Cu– β -glucan-mediated disease resistance

Taken together, the present findings support a model in which CN–Cu– β -glucan nanoconjugate treatment establishes a broad biochemical defence state in tuberose. The chitosan component provides a recognized elicitor capable of stimulating plant immune responses, the β -glucan component supplies an additional carbohydrate-derived defence stimulus, and Cu potentially contributes to essential enzymatic and photosynthetic functions. Evidence for chitosan/chitooligosaccharide-mediated immune perception has been established experimentally in grapevine (Brulé *et al.*, 2019) [16], while β -glucan elicitors have independently been shown to trigger defence enzymes and disease resistance (Shinya *et al.*, 2006; Aziz *et al.*, 2003) [17, 18]. The resulting phenotype in tuberose was characterized by simultaneous enhancement of hydrolytic defence (β -glucanase and chitinase), phenolic-associated defence (PPO and total phenolics), and physiological maintenance (chlorophyll), accompanied by profound suppression of disease. Chitosan nanoparticle studies in tomato and chilli similarly demonstrate that nanoparticulate chitosan can induce defence-related genes, enzymes and signalling responses associated with enhanced pathogen resistance (Chun *et al.*, 2019; Sarkar *et al.*, 2022) [15, 22]. The present study extends this concept by demonstrating a coordinated biochemical response to a CN–Cu– β -glucan nanoconjugate in tuberose.

The particularly strong PPO response should also be considered in relation to Cu because PPOs are copper metalloenzymes (Zhang *et al.*, 2021) [19], while Cu itself is integral to photosynthetic and enzymatic processes (Wang *et al.*, 2024) [20]. Thus, the CN–Cu– β -glucan formulation may simultaneously provide elicitor-derived defence stimulation and a Cu-associated metabolic contribution.

Overall, the data indicate that 100–125 ppm CN–Cu– β -glucan represents the effective response range under the present pot-culture conditions, with 100 ppm being particularly notable because it produced the highest or near-highest values for several defence parameters while remaining statistically at par with 125 ppm for the principal responses. This distinction is important because increasing nanoparticle concentration beyond the effective range does not necessarily result in

additional biological benefit. The findings therefore provide a rational basis for selecting approximately 100 ppm for subsequent greenhouse and field validation.

5. Conclusion

The present study demonstrates that the CN–Cu– β -glucan nanoconjugate can substantially modify the biochemical and pathological status of tuberose under pot-culture conditions. Its effectiveness was characterized not only by strong suppression of disease but also by coordinated enhancement of multiple host defence-associated traits. The 100 ppm treatment (T_6) recorded the highest β -glucanase and chitinase activities, with increases of 53.49 and 77.27%, respectively, over the untreated control, while the 125 ppm treatment (T_7) remained statistically at par. The nanoconjugate also markedly stimulated PPO activity, increased total phenolic content, and maintained or enhanced chlorophyll concentration, indicating that disease protection was accompanied by preservation of important physiological functions.

The pathological response was particularly pronounced: disease incidence decreased from 91.67% in untreated plants to complete absence of disease at both 100 and 125 ppm, giving 100% reported disease control. Because T_6 and T_7 were statistically at par for the major response parameters, the data indicate an effective concentration plateau around 100 ppm rather than a proportional benefit from further concentration increase. The improved response of the CN–Cu– β -glucan formulation at 100 and 125 ppm compared with the individual chitosan- and β -glucan-nanoparticle treatments further supports its potential as a combined multifunctional formulation.

Collectively, the results indicate that the CN–Cu– β -glucan nanoconjugate can integrate disease suppression, activation of hydrolytic and phenolic defence metabolism, and maintenance of photosynthetic pigments within a single treatment strategy. Among the concentrations evaluated, 100 ppm represents the most rational candidate concentration for subsequent validation, given its strong and statistically comparable performance with 125 ppm. Field-scale experiments across seasons, together with pathogen quantification, defence-gene expression, redox/signalling measurements and formulation-dose optimization, will be important to establish the consistency, mechanism and practical applicability of this nanoconjugate for sustainable tuberose disease management.

Acknowledgments

The authors gratefully acknowledge the Department of Plant Pathology, Government College of Agriculture, Pune, for laboratory facilities; AICRP on Floriculture, ZARS, Ganeshkhind, Pune, for pot culture facilities; and Vasantdada Sugar Institute (VSI), Manjari Budruk, Pune, for biochemical analysis facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

Table 1. Treatment details and application schedule of the pot culture experiment.

Sr. No.	Treatment	Treatment details
T_1	Copper oxychloride	Copper oxychloride 0.2% drenching at 40 and 50 DAP
T_2	Chitosan nanoparticles	Bulb dipping followed by foliar spray with chitosan nanoparticles (50 ppm) at 30, 45 and 60 DAP
T_3	β -Glucan nanoparticles	Bulb dipping followed by foliar spray with β -glucan nanoparticles (50 ppm) at 30, 45 and 60 DAP
T_4	CN–Cu– β -glucan nanoconjugate	Bulb dipping followed by foliar spray with CN–Cu– β -glucan nanoconjugate (50 ppm) at 30, 45 and 60 DAP

(continued on next page)

Sr. No.	Treatment	Treatment details	Sr. No.	Treatment	Treatment details
T ₅	CN–Cu–β-glucan nanoconjugate	Bulb dipping followed by foliar spray with CN–Cu–β-glucan nanoconjugate (75 ppm) at 30, 45 and 60 DAP	T ₉	<i>Pseudomonas fluorescens</i>	Bulb dipping followed by soil drenching with <i>Pseudomonas fluorescens</i> at 40 DAP
T ₆	CN–Cu–β-glucan nanoconjugate	Bulb dipping followed by foliar spray with CN–Cu–β-glucan nanoconjugate (100 ppm) at 30, 45 and 60 DAP	T ₁₀	<i>Bacillus subtilis</i>	Bulb dipping followed by soil drenching with <i>Bacillus subtilis</i> at 40 DAP
T ₇	CN–Cu–β-glucan nanoconjugate	Bulb dipping followed by foliar spray with CN–Cu–β-glucan nanoconjugate (125 ppm) at 30, 45 and 60 DAP	T ₁₁	Untreated control	No treatment was applied
T ₈	<i>Trichoderma viride</i>	Bulb dipping followed by soil drenching with <i>Trichoderma viride</i> at 40 DAP	Note: DAP, days after planting; CN–Cu–β-glucan, chitosan–copper–β-glucan nanoconjugate.		

Table 2. Effect of different treatments on defence-related biochemical parameters and chlorophyll a and b contents in tuberos leaves.

Treatment	β-Glucanase activity (U mg ⁻¹ protein)	Chitinase activity (U mg ⁻¹ protein)	Total phenol (mg g ⁻¹ FW)	PPO activity (Δ Abs min ⁻¹ g ⁻¹ FW)	Chl a (mg g ⁻¹ FW)	Chl b (mg g ⁻¹ FW)
T ₁	0.39 ^d	1.00 ^{bc}	5.64 ^{cd}	0.12 ^{de}	0.14 ^{abc}	0.10 ^{abcd}
T ₂	0.44 ^{cd}	1.06 ^{bc}	5.83 ^{ab}	0.17 ^{bcd}	0.13 ^c	0.09 ^d
T ₃	0.45 ^{cd}	1.09 ^{bc}	5.70 ^{bc}	0.19 ^{abc}	0.15 ^{ab}	0.10 ^{abc}
T ₄	0.50 ^{bcd}	1.12 ^{bc}	5.74 ^{abc}	0.16 ^{cd}	0.15 ^{ab}	0.11 ^a
T ₅	0.56 ^{abc}	1.29 ^{ab}	5.82 ^{ab}	0.24 ^{ab}	0.16 ^a	0.11 ^{ab}
T ₆	0.66 ^a	1.56 ^a	5.91 ^a	0.24 ^a	0.16 ^a	0.11 ^a
T ₇	0.62 ^{ab}	1.45 ^a	5.90 ^a	0.20 ^{abc}	0.16 ^a	0.11 ^a
T ₈	0.40 ^d	0.97 ^c	5.79 ^{abc}	0.20 ^{abc}	0.14 ^{bc}	0.10 ^{bcd}
T ₉	0.51 ^{bcd}	1.15 ^{bc}	5.81 ^{abc}	0.16 ^{cde}	0.13 ^{bc}	0.10 ^{cd}
T ₁₀	0.41 ^d	0.88 ^c	5.80 ^{abc}	0.15 ^{cde}	0.13 ^{bc}	0.10 ^{cd}
T ₁₁	0.43 ^{cd}	0.88 ^c	5.51 ^d	0.09 ^e	0.14 ^{bc}	0.10 ^{cd}
CD (0.05)	0.145	0.294	0.182	0.067	0.021	0.014
SE(m)±	0.049	0.100	0.062	0.023	0.007	0.005

Note: Means with at least one common letter are not significantly different at the 5% level (protected Fisher LSD). FW, fresh weight; PPO, polyphenol oxidase; Chl, chlorophyll; CD, critical difference; SE_m, standard error of mean.

Table 3. Effect of different treatments on total chlorophyll, carotenoid content, chlorophyll a/b ratio, stem rot disease incidence and disease control in tuberos.

Treatment	Total chlorophyll (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)	Chl a/b (ratio)	Disease incidence (%)	Disease control (%)
T ₁	0.24 ^{bcd}	0.01 ^{bcd}	1.44 ^a	50.00 ^{bc}	44.44 ^{cd}
T ₂	0.22 ^e	0.01 ^e	1.43 ^a	33.33 ^{cd}	63.89 ^{bc}
T ₃	0.25 ^{abcd}	0.01 ^{abcd}	1.46 ^a	33.33 ^{cd}	63.89 ^{bc}
T ₄	0.26 ^{abc}	0.01 ^{abc}	1.35 ^a	25.00 ^d	72.22 ^b
T ₅	0.27 ^{ab}	0.01 ^{abc}	1.44 ^a	16.67 ^{de}	80.56 ^{ab}
T ₆	0.27 ^{ab}	0.01 ^{ab}	1.42 ^a	0.00 ^e	100.00 ^a
T ₇	0.28 ^a	0.01 ^a	1.46 ^a	0.00 ^e	100.00 ^a
T ₈	0.23 ^{cde}	0.01 ^{cde}	1.41 ^a	58.33 ^b	36.11 ^d
T ₉	0.23 ^{de}	0.01 ^{cde}	1.41 ^a	66.67 ^b	27.78 ^d
T ₁₀	0.23 ^{de}	0.01 ^{abcd}	1.39 ^a	66.67 ^b	25.00 ^d
T ₁₁	0.23 ^{cde}	0.01 ^{de}	1.42 ^a	91.67 ^a	0.00 ^e
CD (0.05)	0.029	0.001	NS	19.611	20.042
SE(m)±	0.010	0.000	0.074	6.648	6.794

Note: Means with at least one common letter are not significantly different at the 5% level (protected Fisher LSD). NS, not significant; FW, fresh weight; Chl, chlorophyll; CD, critical difference; SE_m, standard error of mean.

(continued on next page)

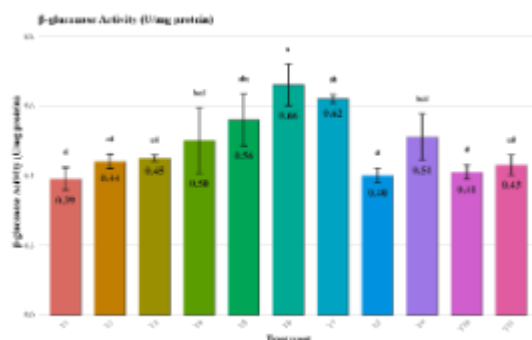


Fig. 2. Effect of different treatments on β -glucanase activity in tuberose.

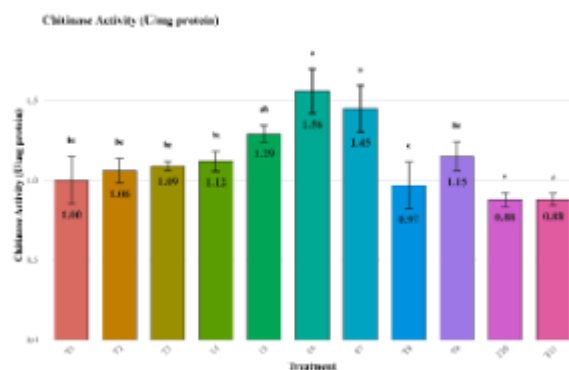


Fig. 3. Effect of different treatments on chitinase activity in tuberose.

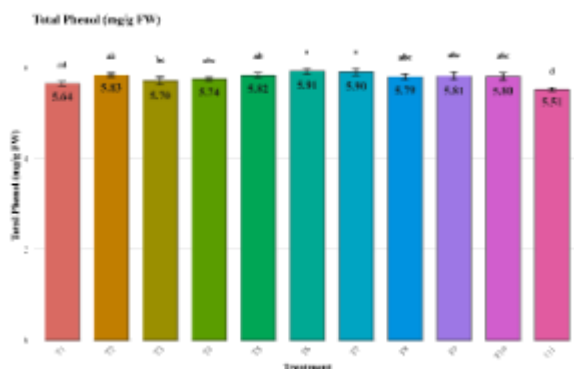


Fig. 4. Effect of different treatments on total phenol content in tuberose.

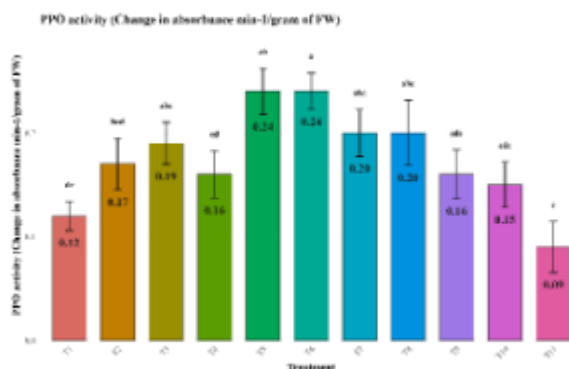


Fig. 5. Effect of different treatments on polyphenol oxidase (PPO) activity in tuberose.

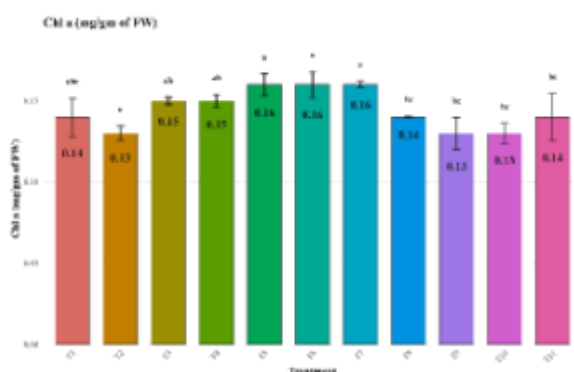


Fig. 6. Effect of different treatments on chlorophyll a content in tuberose.

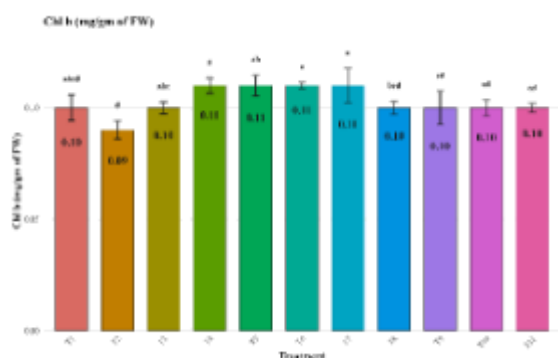


Fig. 7. Effect of different treatments on chlorophyll b content in tuberose.

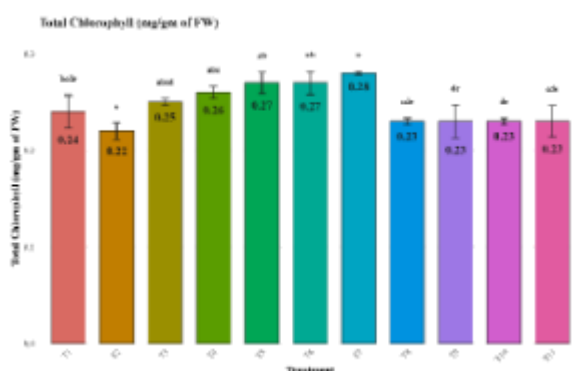


Fig. 8. Effect of different treatments on total chlorophyll content in tuberose.

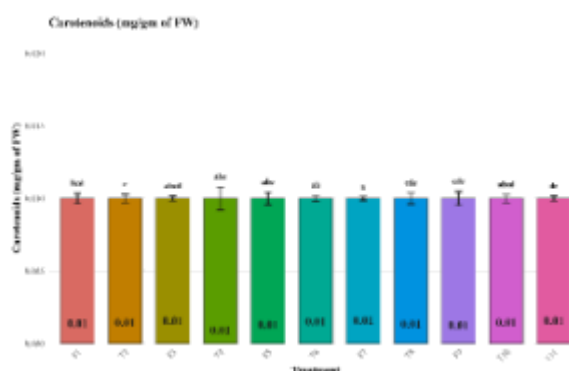


Fig. 9. Effect of different treatments on carotenoid content in tuberose.

(continued on next page)

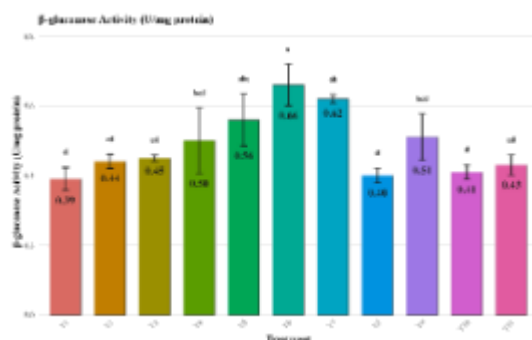


Fig. 2. Effect of different treatments on β -glucanase activity in tuberose.

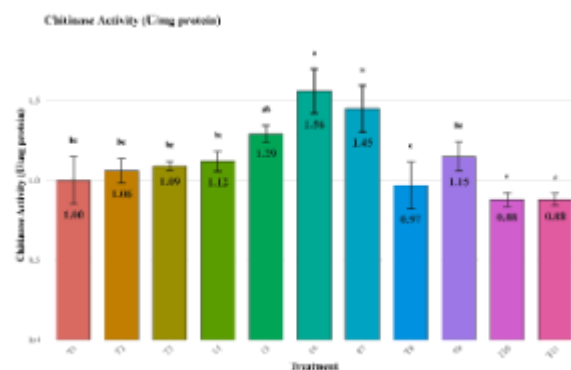


Fig. 3. Effect of different treatments on chitinase activity in tuberose.

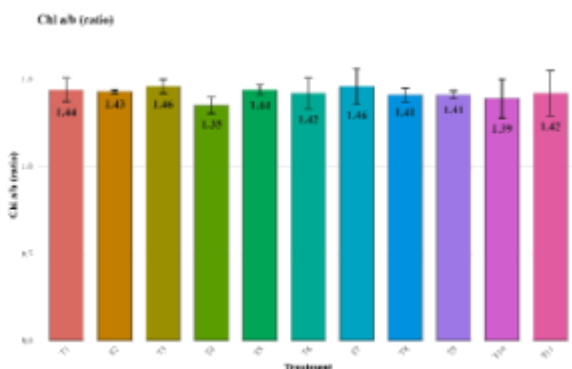


Fig. 10. Effect of different treatments on chlorophyll *a/b* ratio in tuberose.

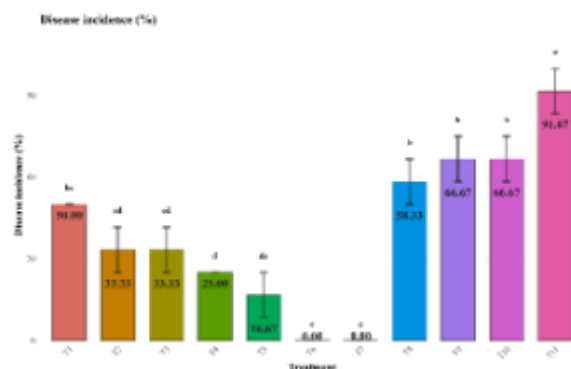


Fig. 11. Effect of different treatments on stem rot disease incidence in tuberose.

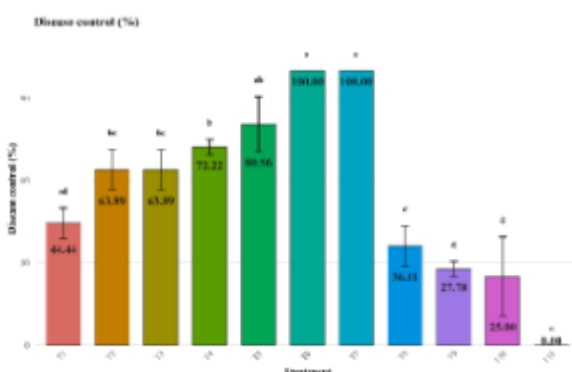


Fig. 12. Effect of different treatments on stem rot disease control in tuberose.

References

- Boopathi P. Morphological and physiological characteristics of *Sclerotium rolfii* causing stem rot or rhizome rot disease in tuberose (*Polianthes tuberosa*). J Crop Weed. 2023;19(1):276-284.
- Wheeler BEJ. An introduction to plant diseases. New York: John Wiley & Sons; 1969.
- Sankara Reddy NH, Sivakumar T, Balabaskar P, Kuralarasi K. Bio-control efficiency of *Pseudomonas fluorescens* against stem rot of tuberose caused by *Sclerotium rolfii*. Plant Arch. 2021;21(1):437-443.
- Sankara Reddy NH, Sivakumar T, Balabaskar P. Bio-control efficiency of *Trichoderma viride* against stem rot of tuberose caused by *Sclerotium rolfii*. Int J Curr Microbiol Appl Sci. 2021;10(11):407-415.
- Chandra S, Chakraborty N, Dasgupta A, Sarkar J, Panda K, Acharya K. Chitosan nanoparticles: a positive modulator of innate immune responses in plants. Sci Rep. 2015;5:15195.
- Saberi Riseh R, Vatankhah M, Hassanisaadi M, Varma RS. A review of chitosan nanoparticles: nature's gift for transforming agriculture through smart and effective delivery mechanisms. Int J Biol Macromol. 2024;260(Pt 2):129522.
- Saberi Riseh R, Gholizadeh Vazvani M, Kennedy JF. β -Glucan-induced disease resistance in plants: a review. Int J Biol Macromol. 2023;253(Pt 4):127043.
- Saberi Riseh R, Gholizadeh Vazvani M, Vatankhah M, Kennedy JF. Chitin-induced disease resistance in plants: a review. Int J Biol Macromol. 2024;266(Pt 1):131105.
- Chavanke SN, Dalvi SG. Biopolymer-engineered novel chitosan-copper- β -glucan nanocomposite: a sustainable antifungal innovation for enhanced plant immunity and crop yield. Int J Biol Macromol. 2026;366:152472.
- Chavanke SN, Dalvi SG. Bioinspired nano-architected chitosan- β -glucan nanocomposite as an elicitor for disease management sustainably. Sci Rep. 2025;15:34031.

11. Chavanke SN, Penna S, Dalvi SG. β -Glucan and its nanocomposites in sustainable agriculture and environment: an overview of mechanisms and applications. *Environ Sci Pollut Res*. 2022;29:80062-80087.
12. Vanti GL, Masaphy S, Kurjogi M, Chakrasali S, Nargund VB. Synthesis and application of chitosan-copper nanoparticles on damping off causing plant pathogenic fungi. *Int J Biol Macromol*. 2020;156:1387-1395.
13. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. New York: John Wiley & Sons; 1984.
14. Panse VG, Sukhatme PV, Amble VN. Statistical methods for agricultural workers. 4th ed. New Delhi: Indian Council of Agricultural Research; 1985.
15. Chun SC, Chandrasekaran M. Chitosan and chitosan nanoparticles induced expression of pathogenesis-related proteins genes enhances biotic stress tolerance in tomato. *Int J Biol Macromol*. 2019;125:948-954.
16. Brulé D, Villano C, Davies LJ, Trdá L, Claverie J, Héloir MC, *et al*. The grapevine (*Vitis vinifera*) LysM receptor kinases VvLYK1-1 and VvLYK1-2 mediate chitooligosaccharide-triggered immunity. *Plant Biotechnol J*. 2019;17(4):812-825.
17. Shinya T, Ménard R, Kozono I, Matsuoka H, Shibuya N, Kauffmann S, *et al*. Novel β -1,3-,1,6-oligoglucan elicitor from *Alternaria alternata* 102 for defence responses in tobacco. *FEBS J*. 2006;273:2421-2431.
18. Aziz A, Poinssot B, Daire X, Adrian M, Bézier A, Lambert B, *et al*. Laminarin elicits defence responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Mol Plant Microbe Interact*. 2003;16(12):1118-1128.
19. Zhang J, Sun X. Recent advances in polyphenol oxidase-mediated plant stress responses. *Phytochemistry*. 2021;181:112588.
20. Wang RX, Wang ZH, Sun YD, Wang LL, Li M, Liu YT, *et al*. Molecular mechanism of plant response to copper stress: a review. *Environ Exp Bot*. 2024;218:105590.
21. Mawale KS, Giridhar P. Chitosan nanoparticles modulate plant growth, and yield, as well as thrips infestation in Capsicum spp. *Int J Biol Macromol*. 2024;254(Pt 1):127682.
22. Sarkar A, Chakraborty N, Acharya K. Chitosan nanoparticles mitigate *Alternaria* leaf spot disease of chilli in nitric oxide dependent way. *Plant Physiol Biochem*. 2022;180:64-73.
23. Sathiyabama M, Manikandan A. Chitosan nanoparticle induced defence responses in finger millet plants against blast disease caused by *Pyricularia grisea* (Cke.) Sacc. *Carbohydr Polym*. 2016;154:241-246.

How to cite this article: J M, Lohate S, Dalvi S, Chavanke S, Karade V, Ghajal L. Chitosan–copper– β -glucan nanoconjugate against *Sclerotium Rolfsii* causing stem rot in tuberose: Disease incidence and defence-related responses. *International Journal of Advanced Biochemistry Research*. 2026;SP-10(10):945-953. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i10SI.10405>