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ORIGINAL ARTICLE

Garlic-Loaded Chitosan Nanoparticles Enhance Dual Antifungal and Defensive properties in Protecting Sugar Beet from *Sclerotium rolfsii*

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ABSTRACT

Sugar beet (*Beta vulgaris* L.) suffers significantly of damping-off and root-rot caused by the soil-borne fungal pathogen *Sclerotium rolfsii* Sacc. (Perfect stage: *Athelia rolfsii* Cruzi.). Chitosan Nano-particles (ChNPs), magnesium Nano-particles (MgNPs), chitosan Nano-particles loaded with garlic (Chitosan at Garlic), and chitosan Nano-particles loaded with algae (Chitosan at algae) were evaluated for their antifungal properties against *S. rolfsii* under greenhouse, and field conditions. In greenhouse studies MgNPs had no discernible effect, whereas ChNPs, Chitosan at Garlic, and Chitosan at algae suppressed (100%) the mycelial growth at 50 µg ml^{-1}. Among the tested Nano-particles, magnesium NPs were the least effective, followed by ChNPs and Chitosan at algae, while greenhouse experiments confirmed that Chitosan at Garlic performed best, resulting in 82.6% healthy roots at 50 µg ml^{-1}. Enzyme activity studies indicated that plants treated with ChNPs and Chitosan at Garlic had significant higher of peroxidase (POD), polyphenol oxidase (PPO), and chitinase, indicating possible improved systemic resistance. In two consecutive seasonal planting, ChNPs at 50 µg ml^{-1}significantly increased root length, fresh weight, dry matter, sucrose content, and total soluble solids, and achieved the maximum disease reduction (82.6 and 71.67%) compared with infected controls and, in some cases, with the fungicide Vitavax 200. By combining direct antifungal activity with activation, these results suggest that chitosan-based Nano-particles especially formulations loaded with garlic Nano provide an environmentally friendly alternative to fungicides for the management of *S. rolfsii* of sugar beet.

Keywords: Biocontrol, Plant defense enzymes, Yield quality, Biosynthesis, Soil-borne pathogens.

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INTRODUCTION

Sugar beet (Beta vulgaris L.) is a major source of sucrose and makes a substantial contribution to the global sugar industry, which typically operates on a biennial cultivation cycle. However, sugar beet production is often constrained by a range of diseases that reduce plant health and yield (Misra *et al*., 2022a). Among these, root-rot diseases are a major concern in meeting processing quality standards, as the sugar industry relies on maximizing sucrose extraction from roots (Misra *et al*., 2022b). The extent of fungal damage is strongly influenced by environmental conditions (Jacobsen, 2006). The crop losses may range from 0.0–50%, but can reach up to 60% or even cause total crop failure (Ithurrart *et al*., 2004; Buhre *et al*., 2009). Soil-borne pathogens, particularly *Sclerotium rolfsii* Sacc. represent some of the most serious constraints on sugar beet productivity. This necrotrophic fungus induces both damping-off and root-rot, resulting in substantial yield and quality losses worldwide (Punja, 1985; Mboup *et al*., 2021). The pathogen is very difficult to control by the traditional methods due to its wide host range, high sclerotia

production, and persistence in the soil (Cilliers *et al*., 2000).

Although fungicides continue to be the major management technique, prolonged use raises serious concerns regarding pathogen resistance, environmental contamination, and residue accumulation in food products (Lamichhane *et al.,* 2016). These limitations underscore the urgent need foreco-friendly and sustainable substitutes capable of control *S. rolfsii* and improve crop health. In this context, Nanotechnology has emerged as a promising tool for plant disease management, offering materials with unique physicochemical properties that enhance plant compatibility and antimicrobial efficacy(Kah*et al.,* 2018). Among these, chitosan Nano-particles (ChNPs) have attracted particular attention due to their strong antifungal activity, biocompatibility, and biodegradability (El-Hadrami *et al.*, 2018; Kong *et al.*, 2010). Chitosan exerts its antifungal effects by interfering with nutrient uptake, disrupting fungal cell wall integrity, and interacting electrostatically with fungal membranes (Kumaraswamy *et al.,* 2018). Furthermore, it acts as an elicitor of plant defense, stimulating the production of antioxidant enzymes and pathogenesis-related proteins (Iriti & Varoni, 2015; Romanazzi *et al.,* 2018).

The incorporation of bioactive plant extracts into Nanoparticle formulations can further enhance their antifungal efficacy. Garlic (*Allium sativum* L.) contains organosulfur compounds, such as allicin and ajoene, which possess potent antifungal properties by inhibiting thiol-containing enzymes and disrupting pathogen metabolism (Ankri & Mirelman, 1999). Similarly, bioactive compounds derived from marine algae contain phenolic and polysaccharide-based structures that exhibit both antimicrobial activity and plant growth-promoting effects (Mabrouki *et al.,* 2020; Khan *et al*., 2009). Encapsulation of these extracts within chitosan Nanocarriers enhances their stability, enables targeted delivery, and ensures sustained release at infection sites (Huang *et al*., 2015).

Despite promising laboratory evidence, few studies have systematically assessed the comparative efficacy of chitosan-based Nano-particles loaded with garlic or algal extracts against *S. rolfsii* in sugar beet in vitro, greenhouse, and field conditions. Nevertheless, there remains limited information on the relative effectiveness of garlic- or algae-loaded chitosan Nano-particles under practical conditions, particularly regarding their capacity to modulate plant defense enzyme systems and improve yield quality traits.

Therefore, the aims of this study were to evaluate the antifungal activity of magnesium Nano-particles (MgNPs), chitosan at garlic, chitosan at algae, and chitosan Nano-particles (ChNPs) against *S. rolfsii* in vitro; assess their effectiveness in controlling damping-off and root-rot under greenhouse and field conditions; examine their influence on plant defense-related enzymes; and investigate their impact on sugar beet yield and quality attributes.

MATERIALS AND METHODS

Seed Source and Preparation

The multigerm sugar beet cultivar (Helios poly), susceptible to fungal root-rot diseases, was provided by the North Delta Sugar Company, Egypt. Seeds were surface-sterilized by soaking in 5% sodium hypochlorite solution for 3 minutes, rinsed twice with sterile distilled water, and then air-dried for one hour.

Fungal Isolation

Sclerotium rolfsii was isolated on PDA medium from sugar beet plants showing wilting, white cottony mycelia, and white to golden sclerotia on roots in fields of Kafr El-Sheikh Governorate, Egypt. Based on identification criteria (Pethybridge *et al*., 2019), the most aggressive isolate (Srk1), previously characterized for aggressiveness (Fatouh, 2012), was selected for the present study.

Nano-materials Source and Preparation

Nano-materials used in this study, including magnesium Nano-particles, chitosan Nano-

particles, and chitosan loaded with algae, were previously synthesized and characterized by (Ahmed *et al.,* 2025). Chitosan loaded with garlic was prepared following the same procedures, and its properties were similarly characterized using FTIR, Zeta potential, and DLS analyses.

Preparation of Garlic Extract and Chitosan at Garlic Nano-particles

Garlic extract was prepared by mixing 20 g of freshly ground garlic with 200 mL of distilled water in a 1:10 (w/v) ratio. The mixture was then heated to 60 °C for six hours with continuous stirring, then filtered sequentially through cheesecloth, Whatman No. 2 filter paper, and a 0.22 µm syringe filter. The resulting extract was further processed using sonication at 750 W for 30 minutes (Kumar *et al.,* 2008; Hamouda *et al.,* 2023).

Chitosan Nano-particles were synthesized using a modified ionic gelation method described by Ahmed *et al.* (2025), in which chitosan was cross linked with STPP anions. For the preparation of chitosan loaded with garlic, 10 mL of STPP solution (1:1 v/v) was mixed with 10 mL of the previously prepared garlic extract (0.22 µm filtered). The mixture was subjected to dropwise addition of chitosan under magnetic stirring at 1200 rpm. The resulting suspension was centrifuged at 10,000 rpm for 10 minutes, and the pellets were washed with deionized water and ultrasonically sonicated for 100 seconds at a 28% pulse ratio. This centrifugation–sonication cycle was repeated five times. Finally, the obtained Nano-formula was freeze-dried and stored in a desiccator for further analysis (Ahmed *et al.,* 2025).

***In Vitro* Antifungal Activity**

The antifungal activity of green Nano-particles against *S. rolfsii*, the causative agents of sugar beet diseases, was investigated *in vitro*. The efficiency of green Nano-particles was assessed at four different green Nano-materials: chitosan, magnesium (MgO), garlic, and green algae, using the food poisoning technique (Kim *et al.* 2012). Three concentrations (25, 37.5, and 50 µg ml^{-1}) were tested, along with the recommended dosage of the chemical fungicide for comparison. Plates inoculated only with the pathogen (without Nano-particles or fungicide) served as the control. Each treatment was replicated four times. All plates were incubated at 28 ± 1 °C for five days, and colony diameter was measured every 48 hours until the control plates reached maximum growth according to Ahmed *et al.,* (2025).

Effect of Seed Treatment with Green Nano-materials Under Greenhouse Conditions:

Preparation of Pathogen Mass Inoculum:

The mass inoculum of ***S. rolfsii*** was prepared following the method of El-Kazzaz *et al.,* (2002). The pathogen was grown in glass bottles containing a sand-to-corn mixture (2:3, w/w). Sorghum seeds were then incubated for 15 days at room temperature (25–27 °C) with a 1 × 2 cm agar plug taken from a 5-day-old culture of ***S. rolfsii*** grown on PDA medium. The resulting inoculum was mixed into sterilized soil at a rate of 2–3% (w/w) to ensure homogeneity. Pots were watered immediately after inoculation.

Pot Experiments:

Pot experiments were conducted as described by Farooq *et al.* (2011). Clay pots (25 cm diameter) were filled with 3 kg of sterilized silt soil. The prepared inoculum was incorporated into the soil at a rate of 2% (w/w). To promote fungal colonization, the inoculated soil was watered and left to settle for one week before planting. Sugar beet seeds (cv. Helios poly) were surface-sterilized and treated with Nano-particle suspensions at three concentrations (50, 75, and 100 µgml-1) containing 0.2% Tween 80. Seeds were soaked for 30 min to planting. Untreated seeds sown in sterile soil served as the negative control, while seeds treated with Vitavax 200 fungicide (3 g/kg) served as the positive control. Ten seeds were sown per pot, and each treatment was replicated in three pots.

Pre- and post-emergence damping-off were recorded 15 and 45 days after planting, respectively. Plants were later thinned to two per pot, and root-rot incidence was assessed 150 days after planting. The percentage of infection was calculated accordingly.

6.3. Disease assessment

(A) Disease assessment was measured as percentage of pre- and post-emergence damping-off after 15 and 45 days (Gouda, 2001), while root-rot incidence after 150 days from sowing, respectively. Percentages of pre- and post-emergence damping-off and root-rot incidence were calculated using the following formula according to (El-Argawy *et al.*, 2016)

% Pre- emergence =

$$\frac{\text{Number of non germinated seeds}}{\text{Number of sown seeds}} \text{X } 100$$

% Post- emergence =

$$\frac{\text{Number of dead seedlings}}{\text{Number of sown seeds}} \text{X } 100$$

% Root-rot =

$$\frac{\text{Number of plants with root - rot}}{\text{Number of sown seeds}} \text{X } 100$$

Field experiments

Field experiment was carried out at Sakha Experimental Research Station in a field known to have the causal pathogen of damping-off *Sclerotium rolfsii* during September 2022 and October 2023 growing seasons. In this experiment, seeds from the sugar beet susceptible cultivar Helios poly were used. Seed were soaked in green Nanomaterial suspensions for 30 minutes prior to planting. The fungicidal impact was compared with Vitavax 200-treated seeds, while untreated seeds served as the control.

The experiment was arranged in a randomized complete block design (RCBD) with three replications. Each plot consisted of three rows (8 m long x 1 m width). Seeds were mechanically sown in hills (two to three seeds per hill) and later thinned to maintain one plant per hill before the next irrigation. Standard agricultural practices, including insect control, fertilization, thinning, irrigation, and other cultural operations, were applied as recommended.

Results were recorded at the end of the 150-day root-rot trial. The percentage of the reduction in root-rot was calculated for each treatment. At harvest, ten plants were randomly selected from each plot to assess quality parameters (total soluble solids, or TSS, and sucrose content), growth and yield components (fresh root weight, dry weight root and humidity).

Determent of quality parameters and yield components:

Root length (cm), fresh weight (g), and dry weight.

After 180 days from the date of seeding, the sugar beet plants were harvested. From each subplot, ten roots were randomly selected, cleaned, and dried to measure root length (cm), fresh weight (g), and dry weight. For dry weight determination, the cleaned roots were cut into small pieces, and a known sample of 100 g was dried in a hot-air oven at 80 °C for 72 hours until a constant weight was obtained.

Yield components and quality

Total soluble solids (TSS) were determined from fresh root juice using a hand refractometer. The sucrose percentage was determined polarimetrically according to the method of Carruthers and Oldfield (1960), using a lead acetate extract prepared from freshly macerated roots.

Biochemical analysis of defense-related enzymes

Samples of sugar beet (*Beta vulgaris* L.) roots that showed the best results after use of Vitavax 200 fungicide, chitosan, magnesium (MgO), garlic, and green algae Nano-particles were collected. Untreated plants were used as the control group. Three replicates were taken in one gram of harvested roots at 14 and 45 days from each treatment were immediately homogenized in liquid nitrogen (Ojha and Chatterjee, 2012). The activities of hydrolytic enzymes (chitinase and β-1,3-glucanase) and the oxidative enzyme (polyphenoloxidase) were determined using crude extracts (Anand *et al.*, 2007).

The enzymatic activities were assayed as follows:

Polyphenoloxidase (PPO) activity:

PPO activity was estimated by mixing 1 mL of enzyme extract with 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 1 mL of 0.1 M catechol as substrate. The increase in absorbance was recorded at 495 nm for 3 min using a spectrophotometer. Enzyme activity was expressed as the change in absorbance min^{-1} g^{-1} fresh weight (Abou-Zeid *et al.,* 2018).

Chitinase activity:

Chitinase activity was assayed using colloidal chitin as substrate. A reaction mixture containing 1 mL of enzyme extract and 1 mL of 1% colloidal chitin in 50 mM sodium acetate buffer (pH 5.2) was incubated at 37°C for 1 h. The released N-acetylglucosamine was measured calorimetrically at 585 nm after reaction with 3,5-dinitrosalicylic acid (DNS). Activity was expressed as µmol N-acetylglucosamine released min^{-1} g^{-1} fresh weight (Abou-Zeid*et al.,* 2018).

β-1,3-glucanase activity:

Enzyme activity was determined using laminarin as substrate. The reaction mixture contained 1 mL of enzyme extract and 1 mL of 0.5% laminarin in 50 mM sodium acetate buffer (pH 5.2). After incubation at 37°C for 30 min, the reducing sugars released were estimated at 585 nm using the DNS method. Activity was expressed as µmol glucose equivalents released min^{-1} g^{-1} fresh weight (Abou-Zeid *et al*., 2018b).

Statistical analysis

The results were examined with one-way ANOVA. Tukey-Kramer test for multiple comparisons at the 0.05 level of significance. The statistical program MINITAB (Minitab R 19.2020.1 version, Minitab Inc., State College, PA, USA) was used.

RESULTS AND DISCUSSION

Characterization of the chitosan loaded with garlic:

Results in Fig (1) showed Chitosan at garlic exhibits a mean hydrodynamic diameter of 73.9 nm with three peaks at 8.3, 22.5 and 396.7 nm; the PDI is 0.497. Similar formulations of chitosan Nano-particles encapsulating garlic extract for seed Nano-priming have been reported to produce spherical particles of approximately200 nm with good storage stability (Mondéjar *et al.,* 2024).

The Zeta potential was 21.6 mV and the conductivity was 1.55 $mScm^{-1}$, which indicates good Nano particle quality. These findings are in line with DosSantos *et al.* (2023), who found A moderately positive zeta potential reflects sufficient colloidal stability due to electrostatic repulsion. This stability is typically attributed to the protonated amine groups of chitosan interacting with negatively charged species, such as garlic bio-activities or solvent residues. As expected, chitosan Nano-particles usually exhibit positive surface charges owing to their amine functionalities, particularly when produced via ionic gelation methods.

FT-IR spectra showed characteristic absorption bands of both chitosan and garlic. The combined profiles exhibited peak shifts and changes in intensity, which indicate physical interactions rather than the formation of new covalent bonds. Istiqomah *et al.,* (2024) similarly reported that, the retention of both chitosan and garlic-specific peaks along with minor shifts or altered intensities is consistent with non-covalent associations such as hydrogen bonding, and ionic interactions. These results suggest that, garlic compounds were effectively entrapped or adsorbed within the chitosan Nano-particles. Such interactions can modify the Nano-particle's surface chemistry and release behavior, which are critical parameters for applications like antimicrobial delivery or bio stimulant seed priming

***In Vitro* Antifungal Activity:**

Different concentrations of four Nano-particles treatments (Magnesium Nano-patricles, Chitosan at garlic, Chitosan and Chitosan at algae) were tested against *S. rolfsii*.

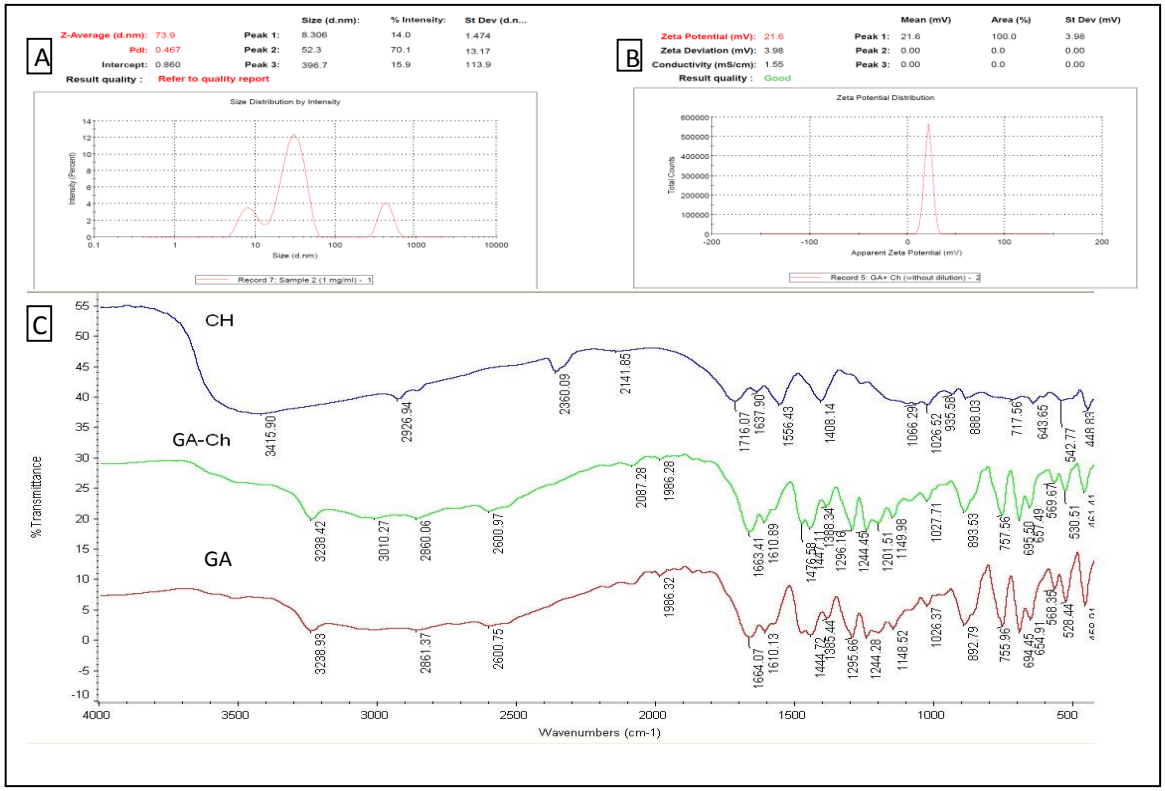

Fig.1. Characterization of Chitosan at garlic (A) Size distribution analysis, (B) Zeta Potential analysis, (C) FTIR spectra analysis.

Data in Table (1) indicated that higher concentrations produced the greatest growth reduction with Chitosan at garlic, Chitosan and Chitosan at algae respectively. Chitosan at garlic was the most effective treatment, recording growth reductions of 55.55, 77.77 and 100 at concentrations of 25, 37.5 and 50µml^{-1}, respectively, followed by Chitosan alone at concentration37.5 and 50 µml-1 showed growth reduction50 and 100 %, respectively. In contrast, Chitosan at algae showed complete inhibition only at 50 µgml^{-1}, while its efficacy at lower concentrations was limited. These results are conforming with earlier findings of Hamouda *et al.*, (2023) and Kumar *et al.*, (2008) who's demonstrated that, plant-derived bioactives combined with Nano-carriers enhance antifungal potency and the strong effect of garlic is likely due to its organosulfur compounds which impair fungal enzymatic systems and oxidative balance.

The antifungal activity of chitosan is well-documented and is largely attributed to its cationic nature, which allows it to interacts with negatively charged microbial cell walls, causing membrane disruption, leakage of cellular contents, and inhibition of nutrient uptake (Kong *et al.*,2010; El Hadrami *et al.*, 2010). The incorporation of garlic extracts likely enhanced antifungal activity through organosulfur compounds (e.g., allicin, ajoene) that impair fungal metabolic enzymes, disrupt thiol-dependent proteins, and induce oxidative stress in fungal cells (Lawson, 1998 and Ankri & Mirelman, 1999;).

Similarly, Chitosan at algae showed complete inhibition at 50 µgml^{-1}, suggesting the presence of bioactive seaweed metabolites (e.g., phenolics, sulfated polysaccharides) with known antifungal properties (Mabrouki *et al.*, 2020). However, its efficacy at lower concentrations was limited, possibly due to a slower release rate

of bioactive compounds compared to garlic-based formulations.

These findings are similar to Kim *et al.* (2012), who reported that combining biopolymers with bioactive plant extracts significantly improved antifungal efficacy compared to individual components. In contrast, the lack of significant inhibition by MgNPs suggests either low ion release under the test conditions or pathogen tolerance, which has also been reported for certain fungi (Jo *et al.*, 2009).

Table 1. Effect of Nano-particles on the redial growth of *S. rolfsii* under laboratory conditions after one week.

Inducer treatments	Concentration (µg ml^{-1})	Redial growth (cm)	Reduction%
ChNP	25	7	22.22
	37.5	4.5	50
	50	0	100
MgNP	25	9	0
	37.5	9	0
	50	9	0
Chitosan at garlic	25	4	55.55
	37.5	2	77.77
	50	0	100
Chitosan at algae	25	9	0
	37.5	6.8	24.44
	50	0	100
Control		9	---
L.S.D. at 5%		**0.423**	**2.341**

Effect of Seed Treatment with Green Nano-materials under Greenhouse conditions

Data in Table (2) showed that Chitosan at garlic significantly decreased both damping-off and root-rot compared to the infected control, with the highest concentration (50 µgml-1) giving ~82.6% healthy roots, followed by ChNP alone which achieved strong control (63.6% healthy roots at 50 µgml^{-1}), but were slightly less effective than garlic-loaded particles. Chitosan at algae was moderately effective, whereas MgNPs showed limited suppression. These greenhouse results confirm the *in vitro* trends and highlight the importance of garlic phytochemicals in enhancing *in vivo* activity. It is widely known that chitosan functions as a plant defense elicitor, activating systemic resistance (ISR) through the pathways of salicylic acid and jasmonic acid (Iriti & Varon, 2015; Romanazzi *et al.*, 2018). Garlic-derived compounds have also been reported to act as elicitors, enhancing peroxidase and polyphenol oxidase activity in infected plants (Gupta & Sharma, 2019). The efficacy of chitosan Nano-particles in controlling soil-borne pathogens such as *S. rolfsii* has been observed in other crops, including tomato and groundnut (Badawy & Rabea, 2011), supporting the potential belief for broad-spectrum application.

Effect of defense-related enzymes in infected roots under in a greenhouse:

Data in Tables (3,4) indicate that Nanomaterial treatments induced notable changes in defense-related enzymes (oxidative and hydrolytic)**.** Peroxidase (POD) and polyphenol oxidase (PPO) significantly increased in infected roots at 14 and 45 days with the highest levels recorded in Chitosan at garlic and ChNP treatments, particularly at 50 µgml^{-1}, indicating activation of host oxidative defense pathways. Chitinase activity, a marker for pathogen cell-wall degradation, was strongly elevated in Chitosan at garlic-treated plants at 45 days, followed by ChNP. While, the

MgNP and algae-based treatments induced minimal enzyme activity, correlating with their lower disease suppression (Table 3). All These results support the conclusion that chitosan-based Nano-materials not only act directly on the pathogen but also prime the plant's systemic resistance in plants.

Table 2. Effect of socking in green Nano-material for sugar beet seeds on damping- off and root - rot diseases caused by *S. rolfsii* under in a greenhouse conditions.

Treatments	Concentration (µg ml^{-1})	% Damping-off		% Root-rot	% Healthy root
		Pre-	Post-		
ChNP	25	10	20	19.05	50.95
	37.5	10	16.67	13.63	59.70
	50	10	13.33	13.05	63.62
MgNP	25	10	23.33	20.00	46.67
	37.5	10	20	19.05	50.95
	50	10	13.33	21.74	54.93
Chitosan at garlic	25	10	6.67	12.00	71.33
	37.5	10	3.33	7.70	78.97
	50	10	0	7.41	82.59
Chitosan at algae	25	10	16.67	22.72	50.61
	37.5	10	13.33	13.05	63.62
	50	10	10	8.33	71.67
Fungicide		10	0	7.41	82.59
Infected control		20	26.67	31.25	22.08
L.S.D. at 5%		**1.042**	**1.835**	**1.836**	**2.469**

POD and PPO are critical oxidative enzymes involved in lignin biosynthesis and phenolic oxidation, which strengthen cell walls and limit pathogen spread (Passardi *et al.*, 2005). Chitinase hydrolyzes fungal cell wall chitin, there by directly contributing to pathogen degradation (Van Loon & Van Strien, 1999). The induction of these enzymes aligns with previous reports that chitosan Nano-particles enhance both oxidative and hydrolytic defense responses in plants (Choudhary *et al*., 2017). The stronger induction observed with garlic-loaded chitosan treatments may result from synergistic elicitation, where garlic's phytochemicals boost reactive oxygen species (ROS) signaling, and further amplify defense gene expression. Similarly, Table (4) shows the effect of different Nano-materials on chitinase activity in sugar beet roots infected with *S. rolfsii* at 14 and 45 days under greenhouse conditions. Results indicate that Chitosan at garlic exhibited the highest induction of chitinase activity, with values reaching 0.0436 and 0.0922 at 50 µgml^{-1}after 14 and 45 days, respectively. This was followed by ChNP, which also showed a marked increase in activity (0.0341 and 0.0682 at 50 µgml^{-1}). These results highlight the strong ability of chitosan-based Nano-materials, particularly when loaded with garlic, to stimulate hydrolytic enzymes involved in pathogen cell wall degradation. consistent with observations in other systems where chitosan Nano-particles or chitosan–garlic composites enhanced antifungal enzyme activity (Olivas-Flores et al., 2024) In contrast, MgNP treatments showed only moderate induction of chitinase, while Chitosan at algae induced negligible activity, remaining close to the infected control. The fungicide produced only slight increases, which were markedly lower than those observed with

Chitosan at garlic (Mondéjar-López *et al.*,2024; Sindhu *et al.*,2023).

Table 3. Activity of peroxidase and polyphenoloxidase in infected roots by *S. rolfsii* after 14 and 45 days

Treatments	Concentration (µg ml^{-1})	Peroxidase activity in the root after (days)		Polyphenoloxidase activity in the root after (days)	
		14	45	14	45
ChNP	25	2.019	1.048	1.772	1.254
	37.5	2.073	1.060	2.111	1.643
	50	2.161	1.681	2.188	1.913
MgNP	25	1.345	1.081	1.050	1.014
	37.5	1.755	1.251	1.216	1.081
	50	1.938	1.273	1.713	1.294
Chitosan at garlic	25	2.141	1.369	2.244	1.811
	37.5	2.231	1.673	2.263	2.011
	50	2.268	2.012	2.819	2.322
Chitosan at algae	25	1.010	0.722	0.541	0.395
	37.5	1.077	0.917	0.647	0.447
	50	1.396	1.021	1.014	0.839
Fungicide		0.711	0.586	0.421	0.300
Infected control		0.312	0.236	0.377	0.218
L.S.D. at 5%		**0.112**	**0.138**	**0.141**	**0.073**

Table 4. Effect of chitinase activity in infected roots by *S. rolfsii* after 14 and 45 days under in a greenhouse condition.

Treatments	Concentration (µg ml^{-1})	Chitinase activity	
		14days	45days
ChNP	25	0.0186	0.0202
	37.5	0.0255	0.0392
	50	0.0341	0.0682
MgNP	25	0.0121	0.0135
	37.5	0.0145	0.0164
	50	0.0178	0.0198
Chitosan at Garlic	25	0.0332	0.0641
	37.5	0.0411	0.0731
	50	0.0436	0.0922
Chitosan at Algae	25	0.0110	0.0101
	37.5	0.0119	0.0114
	50	0.0122	0.0128
Fungicide		0.0088	0.0121
Infected Control		0.0045	0.0056
L.S.D. at 5%		**0.009**	**0.001**

Effect of Seed Treatment with Green Nano materials under Field Conditions:

In field experiments (Table 5), Chitosan at Garlic at 50 µgml-1 achieved the highest disease decrease (77.1% in 2022; 69.8% in 2023), in some cases outperforming the fungicide Vitavax 200. Also, ChNP and Chitosan at algae also significantly decrease root-rot but with slightly lower efficacy, while MgNP remained the least effective Nanomaterial under natural field conditions as shown in table (5).

These field results are in close agreement with those obtained under greenhouse conditions, which demonstrating the reliability and reproducibility of the tested treatments. This consistency across controlled and natural environments highlights the robustness of the observed effects and supports their potential applicability under practical agricultural conditions. such field-level disease suppression by chitosan/garlic-based Nano-materials aligns with recent observations: for instance, Mondéjar-López et al. (2024) demonstrated that polymeric Nano-particles of garlic extract–chitosan effectively reduced disease in cereal field trials. Also, chitosan Nano-particles have been widely reported to function not only as direct antimicrobial agents but also as elicitors of plant defense responses under field conditions (Nandhini, et al. 2025).

Table 5. Efficiency of fungicide and some green Nano-material on sugar beet root-rot *S. rolfsii* and crop yield under field condition (Sakha, 2023 \ 2024).

Treatment	Concentration	% disease incidence		% decrease	
		2023	2024	2023	2024
ChNP	25	23.83	21.18	54.44	58.35
	37.5	28.73	22.7	45.07	55.36
	50	25.27	21.8	50.73	57.53
MgNP	25	30.58	32.05	41.54	36.98
	37.5	29.90	27.77	42.84	45.39
	50	26.81	22.55	48.74	55.66
Chitosan at Garlic	25	23.38	21.18	55.30	58.35
	37.5	16.66	16.00	68.15	68.54
	50	11.97	15.34	77.11	69.83
Chitosan at Algae	25	30.58	32.33	41.54	36.43
	37.5	36.28	32.44	30.64	36.21
	50	19.91	18.28	61.93	64.05
Fungicide		18.14	18.8	65.32	63.03
Control		52.31	50.86	0.0	0.0
L.S.D. at 5%		**1.016**	**0.879**	**1.072**	**1.099**

Effect of Seed Treatment with Green Nano materials on quality parameters and yield components under field conditions:

Data in Tables (6,7) showed that Chitosan at Garlic treatments, particularly at higher concentrations, significantly improved root length, fresh weight, and dry matter content, with clear consistency across both seasons. This was accompanied by increased sucrose content (up to 19.6%) and TSS (up to 24.5%), surpassing not only the infected controls but also the fungicide-treated plots. Chitosan at algae also enhanced TSS and sucrose levels at higher concentrations, suggesting that algae-derived metabolites may contribute to sugar accumulation. These findings align with Romanazzi *et al.*, (2018), who reported that chitosan-based formulations in field crops enhanced both disease control and yield parameters,

supporting the dual role of Nanostructured chitosan as a protectant and a growth promoter. Chitosanatalgae's improvement in TSS is further supported by Khan *et al*. (2009), who attributed such effects to the biostimulant activity of seaweed-derived polysaccharides on carbohydrate metabolism.

Table 6. Effect of green Nanomaterial on plant growth parameter of sugar beet rot *S. rolfsii* under field conditions.

Treatments	Concentration (µg ml^{-1})	Root length (m) Season		Fresh weight (kg) Season		Dry weight\ 100 (g) Season		Humidity % Season	
		2023	2024	2023	2024	2023	2024	2023	2024
ChNP	25	24.6	23.8	1250	1283	68.14	70.69	31.86	29.31
	37.5	23	22.8	1115	1100	38.05	34.83	61.5	65.17
	50	22.6	22	1233.33	1250	68.14	64.48	31.86	35.52
MgNP	25	20.3	18.6	966.66	916.66	30.23	25.02	69.77	74.98
	37.5	.523	23	1166.66	1050	29.69	29.18	70.31	70.82
	50	24	24.5	1200	1100	68.14	64.48	31.86	35.52
Chitosan at Garlic	25	26.9	25.8	1250	1283	74.2	70.69	25.8	29.31
	37.5	31.4	30.5	1833	1500	75.06	73.73	25	26.27
	50	36.7	34.5	2083.33	2166	90.02	77.67	10	22.33
Chitosan at Algae	25	20.8	19.4	983.33	966.66	24.5	25.02	75.5	74.98
	37.5	22	21.3	1000	983.33	25.02	18.49	74.98	81.51
	50	29.3	28.8	1433	1416	75.06	73.73	24.94	26.27
Fungicide		20.2	19.5	983.33	1000	46.02	49.28	49.28	50.72
Infected Control		19	18	766.66	733.33	34.78	34.49	34.49	65.51
Non-infected Control		27	26.6	1200	1100	75.06	73.72	73.72	26.28
L.S.D. at 5%		**4.703**	**4.428**	**4.867**	**4.372**	**3.979**	**4.251**	**1.835**	**1.765**

The overall experimental pattern indicates that ChNP and Chitosan at Garlic consistently outperformed other treatments in both pathogen suppression and growth enhancement through a combination of direct antifungal action, structural damage to fungal hyphae, and stimulation of host defense enzymes, Chitosan Nano-particles have been shown to cause distortion of hyphal structure and reduce spore germination, while also upregulating enzymes such as chitinases, peroxidases, etc. (Khaledi et al. 2015; Iqbal, et al. 2024) These results, observed under both greenhouse and field conditions, highlight the strong potential of chitosan-based Nano-materials—particularly Chitosan at Garlic—as practical, eco-friendly alternatives to chemical fungicides, capable of simultaneously decrease disease pressure and enhancing crop yield and quality, similar consistency has been reported under field trials where chitosan treatments reduced disease severity and enhanced growth parameters compared to controls and sometimes surpassed fungicide treatments (Debnath et al. 2018).

CONCLUSION

The results showed that chitosan-based Nano-particles, especially those loaded with garlic, are very effective and environmentally friendly substitutes for synthetic fungicides against *S. rolfsii* in sugar beet. Their dual action of direct antifungal toxicity and activation of plant defense enzymes makes them attractive options for integrated disease management (IDM) programs. Lastly, field data confirms that Nano-materials can maintain performance under natural disease pressure, with favorable effects on yield quality traits.

Table 7. Effect of green Nano-material and fungicide on sucrose % and T.S.S % in rotted beet roots under field condition.

Treatments	Concentration (µg ml^{-1})	Season 2023		Season 2024	
		Sucrose	T.S.S	Sucrose	T.S.S
ChNP	25	15.60	19.50	15.55	14.43
	37.5	14.44	18.50	14.50	18.12
	50	14.80	18.50	14.60	18.25
MgNP	25	12.60	15.75	11.85	14.81
	37.5	14.4	18.00	14.20	17.75
	50	15.00	18.75	14.90	18.62
Chitosan at Garlic	25	15.60	19.50	15.50	18.75
	37.5	17.39	21.73	17.07	21.33
	50	19.60	24.50	19.00	23.75
Chitosan at algae	25	12.95	16.18	12.88	16.10
	37.5	13.80	17.25	13.65	17.06
	50	18.50	23.12	18.43	23.03
Fungicide		15.60	19.50	15.40	19.25
Infected Control		12.40	15.50	11.50	14.37
Non-infected Control		14.40	18.00	19.20	24.00
L.S.D. at 5%		3.60	3.20	73.00	3.847

AUTHOR CONTRIBUTIONS

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

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