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RESEARCH ARTICLE

Integrated Management of Cucumber Damping-Off Disease Caused by the Fungus *Rhizoctonia solani* using Some Environmentally Friendly Agents.

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ABSTRACT

This research aimed to evaluate integrated management factors for cucumber damping-off disease caused by the pathogenic fungus *Rhizoctonia solani*, and to identify sustainable alternatives based on the principle of synergism between biological and nanotechnological agents to reduce reliance on chemical pesticides. All field and laboratory experiments were conducted at the College of Agriculture, University of Karbala. The methodology relied on isolating the pathogen and testing the efficacy of the biocontrol fungus *Trichoderma viride*, nano-chitosan particles (Nano-Chitosan), and chelated mineral elements (iron and manganese), by reducing the dose of the chemical pesticide (Tolclofos-methyl) to half and testing this integrated combination in vitro and in the field. The main results showed the pathogen's high capacity to reduce seed germination to 28.5%, whereas the integrated combination (the proposed model) achieved complete inhibition of pathogen growth in vitro. In field experiments, seedling survival increased to 95.6% compared with 22.5% under pathogen infection alone. The results also recorded a qualitative increase in shoot dry weight of 333%, confirming the activation of the plant's systemic defensive response. It is concluded that the multi-pathway attack technique that integrates biological and nano agents provides effective and sustainable protection for the crop while reducing chemical inputs by 50%. The findings of this research recommend adopting this integrated model as a fundamental pillar in integrated pest management (IPM) programs to ensure environmentally friendly agricultural production capable of efficiently confronting soil-borne pathogens with high effectiveness.

Keywords: *Rhizoctonia solani, Trichoderma viride, Nano-Chitosan, Seedling damping-off, Integrated control*

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is considered one of the most important economic vegetable crops belonging to the cucurbit family (Cucurbitaceae) worldwide. It is characterized by high nutritional value, as it is a rich source of vitamins, minerals, and antioxidants necessary for human health, and is also a fundamental pillar in the protected and open-field cultivation sector. Global statistics indicate a steady growth in the production of this crop, led by major countries. At the local level, cucumber is one of the

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strategic crops in the Iraqi food basket, for which large areas are allocated to meet the increasing demand in local markets (FAOSTAT, 2020; AL-Rikabi *et al.,* 2021). Despite the economic importance of the crop, global and local production faces acute challenges that lead to a noticeable decline in both quantity and quality. These reductions are attributed to the interaction of several environmental and biological factors; foremost among them are soil-borne fungal pathogens (Mirzwa-Mróz *et al.,* 2024).

The fungus *Rhizoctonia solani* is one of the most dangerous pathogens attacking the crop in its early stages (Almaghasla *et al.,* 2024). The danger of this fungus lies in causing damping-off disease and root rot, as scientific reports indicate that infection with *R. solani* may cause a sharp decline in seed germination rates and yield deterioration by proportions that may exceed 70% in cases of severe infection, leading to complete failure in crop establishment (Abd Al-Qader *et al.,* 2024). Farmers have long relied on chemical pesticides as the first option to control this fungus, but the intensive use of traditional pesticides has been associated with complex environmental and health problems; it has led to the contamination of soil and water sources, the emergence of resistant fungal strains, as well as toxic risks that threaten human and animal health and disturb the biological balance of plants (Islam *et al.,* 2024).

In response to these risks, there is an urgent need to adopt environmentally friendly and sustainable strategies, the most important of which is the use of biocontrol agents such as *Trichoderma viride*, which has proven exceptionally efficient in suppressing plant pathogens through parasitic and competitive mechanisms, without leaving any harmful side effects (Raman *et al.,* 2024) .

Based on the above, the current research aims to evaluate the synergistic effectiveness of the use of chelated mineral elements (iron and manganese) and nano- chitosan in combination with the biological fungus *T. viride* in controlling the disease of damping-off caused by the fungus *R. solani*, to provide an integrated control model that reduces dependence on traditional chemical pesticides and preserves the safety of the ecosystem.

MATERIALS AND METHODS

Isolation of fungi from inside the plant

Samples were collected from the roots of some cucumber plants, and pathogenic fungi were isolated from them. A general weakness in growth was observed in these plants, characterized by yellowing, stunting, and wilting, especially of the older leaves, as well as drying and rotting of the roots, and their turning brown. The samples were taken from fields in Karbala and Babil governorates in the desert area, and from the plastic house of the Faculty of Agriculture/ University of Karbala. The samples were placed in plastic bags and brought to the postgraduate laboratory in the Plant Protection Department/ College of Agriculture/ University of Kerbala to perform the isolation process. The roots were washed under tap water for about 30 minutes to remove soil, and then cut into small pieces and surface-sterilized with sodium hypochlorite solution (1%) for two minutes, and after that, they were rinsed with distilled water three times. The plant pieces were dried with sterile filter paper, transferred to petri dishes containing potato medium dextrose Ager (PDA) equipped with the antibiotic Chloramphenicol, and incubated at a temperature of 25 ± 2°C for 3 days. Fungi were initially diagnosed based on their phenotypic characteristics and using taxonomic keys described by Ellis et al. (2007), Watanabe (2002) and Leslie and Summerell (2006).

Testing the pathogenicity of the isolated fungi

Testing the pathogenicity of the isolated fungi against the germination of cucumber seeds on Water Agar medium

The pathogenicity of the fungi isolated in this study from the roots of infected cucumber plants was tested based on the method described by Bolkan and Butler (1974), "by inoculating Petri dishes (8.5 cm) containing about 20 ml of water agar medium (Water Agar) supplemented with the antibiotic tetracycline at a rate of 250 mg/L of culture medium after sterilization by autoclave. The center of each dish was inoculated with a disc (0.5 cm) taken from a 7-day-old fungal colony and incubated at 25 ± 2°C for three days. After that, cucumber seeds (local) surface-sterilized with sodium hypochlorite (NaClO, 1%) were planted in a circular arrangement at a rate of 10 seeds/dish. A control treatment was also carried out using the same method mentioned above, except that no fungus was inoculated, with three replicates per treatment (dishes).

All plates were placed in a 25 ± 2 °C incubator until all seeds in the control

treatment had germinated. Afterward, the percentage of seed germination was calculated from the following equation:

Germination percentage = (number of germinated seeds / total number of seeds) × 100

Testing the pathogenicity of fungal isolates on cucumber seed germination in plastic pots

This experiment was conducted in the lath house of the College of Agriculture/University of Karbala after mixing loamy soil and peat moss (2:1) and sterilizing the mixture by autoclaving at 121°C and 15 lb/ in² for 60 minutes, with a repeat on the second day. Thereafter, the fungal inoculum (1%) was loaded onto the millet seeds and mixed well before placing them in plastic bags and mixing them thoroughly with the soil, then the bags were placed in plastic pots at a rate of kg/pot. All pots were carefully irrigated and covered for 2 days with perforated polyethylene bags. A control treatment was also conducted by following the same steps as before, except that the soil was not contaminated with any fungus. After that, all pots were planted with cucumber seeds (local) (10 seeds/pot) surface-sterilized with sodium hypochlorite solution (NaClO, 1%) and irrigated carefully with repeated irrigation whenever needed. After 24–28 days from sowing, the percentage of seed germination was calculated by using the following equation:

Germination percentage = (number of germinated seeds / total number of seeds) × 100

Based on the results of this experiment, one of the most pathogenic isolates was selected and identified morphologically and molecularly for use in subsequent experiments.

Testing the antagonistic ability of T. viridae isolates against the pathogenic fungus R. solani on PDA medium.

Following the dual culture technique, the antagonistic ability of five isolates of the biocontrol fungus *T. viridae* against the pathogenic fungus *R. solani* was tested on culture medium. A Petri dish (8.5 cm) was divided by an imaginary line into two halves. The center of the first half was inoculated with a disc (0.5 cm) taken from the margin of a 7-day-old colony of the biocontrol fungus, and the center of the other half was inoculated in the same manner with a disc taken from a colony of the pathogenic fungus *R. solani*, with three replicates per treatment. A control treatment was also carried out by inoculating other Petri dishes with the pathogenic fungus only. All plates were kept at 25 ± 2°C until the pathogenic fungus grew to the rim of the plate. Then, the percentage of the efficiency of resistance fungi in inhibiting the growth of pathogenic fungi was calculated by adopting the Abbott equation (1925), proven below:

$$\text{Inhibition percentage} = \frac{\text{fungal growth rate in the control} - \text{fungal growth rate in the treatment}}{\text{fungal growth rate in the control}} X\ 100$$

The degree of biocontrol dominance of the biocontrol fungus was also evaluated based on the scale developed by Bell et al. (1982), which consists of five grades (1–5) as follows:

Grade (1): The biocontrol fungus grows over the pathogenic fungus and covers the entire surface of the culture medium (complete dominance).

Grade (2): The biocontrol fungus covers of the surface area of the culture medium.

Grade (3): The biocontrol fungus and pathogenic fungus grow evenly, and each takes up half the area of the dish.

Grade (4): The pathogenic fungus covers of the surface area of the culture medium and suppresses the growth of the biocontrol fungus.

Grade (5): The pathogenic fungus grows over the biocontrol fungus and covers the entire surface of the culture medium.

Testing the efficacy of chelated iron (Fe-EDTA) and chelated manganese (Mn-EDTA) at different concentrations on the growth of *T. viridae* and the pathogenic fungus *R. solani* (cm)

This experiment was conducted using different concentrations (50, 100, 200, and 1.00 ppm) of chelated iron (Fe-EDTA) and chelated manganese (Mn-EDTA) by adding each separately to sterilized potato dextrose agar (PDA) medium supplemented with the antibiotic chloramphenicol (50 mg/L). Upon pouring and solidifying the medium, a 0.5 cm disc from a fungal culture was inoculated at the center of each Petri dish, with three replicates each for the fungi *T. viridae* and the pathogenic fungus *R. solani*. A control treatment involved inoculating dishes that received no mineral elements. The Petri dishes were then placed in an incubator at 25 ± 2°C. Radial growth of the fungi was monitored, and mean diameters were calculated once the fungal isolate reached the edge of the dish.

The percentage efficiency of the mineral elements in inhibiting the growth of the pathogenic fungus was calculated using Abbott's equation (1925), shown below:

$$\text{Inhibition percentage} = \frac{\text{fungal growth rate in the control} - \text{fungal growth rate in the treatment}}{\text{fungal growth rate in the control}} X\ 100$$

Testing the efficacy of nano-chitosan at different concentrations on the growth of the fungus T. viridae and the pathogenic fungus R. solani (cm)

This experiment was conducted using different concentrations (50, 100, 200, and 100 ppm) of nano-chitosan prepared by the ionic gelation method: chitosan powder was dissolved in a 1% acetic acid solution under magnetic stirring for 24 hours to ensure complete homogenization. After that, a sodium tripolyphosphate (TPP) solution was added, leading to the formation of nanoparticles due to electrostatic interactions between the positive charges of chitosan and the negative charges of TPP. After pouring and solidifying the medium, a disc (0.5 cm) from a fungal culture was placed at the center of each Petri dish for inoculation., with three replicates for each of the fungus *T. viridae* and the pathogenic fungus R. solani. A control treatment was carried out by inoculating other dishes not treated with nano-chitosan. The dishes were incubated in an incubator at 25 ± 2°C. When the fungal isolate in one of the treatments reached the edge of the dish, the results were recorded by calculating the mean radial growth diameters of the growing fungi.

After pouring and solidifying the medium, a disc (0.5 cm) from a fungal culture was placed at the center of each Petri dish for inoculation.

The efficiency percentage in inhibiting the growth of the pathogenic fungus was determined utilizing Abbott's (1925) equation, shown below:.

Inhibition percentage = [(fungal growth rate in the control − fungal growth rate in the treatment) / fungal growth rate in the control] × 100

Testing the chemical pesticide (Tolclofos-methyl 50%) to compare its efficiency with biological and nano alternatives at different concentrations on the growth of the fungus *T. viridae* and the pathogenic fungus *R. solani* (cm)

This experiment was conducted using different concentrations (100, 500, and 0100 ppm) of the fungicide (Tolclofos-methyl 50%). After pouring and solidifying the medium, a disc (0.5 cm) from a fungal culture was placed at the center of each Petri dish for inoculation, with three replicates for each of the fungi *T. viridae* and *R. solani*. A control treatment was carried out by inoculating other dishes not treated with the fungicide. The plates were placed in an incubator set at 25 ± 2°C. Upon reaching the edge of the plate in one of the treatments, the fungal isolate›s progress was measured by determining the average radial growth diameter.

The efficiency percentage in inhibiting the growth of the pathogenic fungus was determined utilizing Abbott's (1925) equation, shown below:

$$\text{Inhibition percentage} = \frac{\text{fungal growth rate in the control} - \text{fungal growth rate in the treatment}}{\text{fungal growth rate in the control}} X\ 100$$

Testing the synergistic interaction study (Synergy Study) between the best selected concentrations of (mineral elements, nano-chitosan, the biocontrol fungus, and half the recommended dose of the pesticide) on the growth of T. viridae isolates and the pathogenic fungus R. solani

To assess the effects of their interaction with mineral elements (chelated iron (Fe-EDTA) and chelated manganese (Mn-EDTA)), nano-chitosan, and chemical pesticide Beltanol on the growth of pathogen fungus *R. solani*, a PDA culture medium was generated in a 1-L glass flask. The medium was then sterilized in an autoclave at and a pressure of 15 lb./in² for 20 minutes. After sterilization and after the temperature decreased to the stage before solidification, and after adding the antibiotic (chloramphenicol), the mineral element particles (chelated iron (Fe-EDTA) and chelated manganese (Mn-EDTA)), nano-chitosan at concentrations (50, 100, 200, and 1000 ppm), and the chemical pesticide at concentrations (50, 250, and 500 ml/L) were added in the same glass flask, then mixed well with the culture medium. A control treatment was carried out without adding the mineral elements or the nanomaterial to the PDA medium. After solidification of the medium, a disc (0.5 cm) from a fungal culture was placed at the center of each, taken from 7-day-old colonies of the fungus *T. viridae* and the pathogenic fungus *R. solani*, with three replicates for each treatment. Dishes were incubated

at 25 ± 2°C and assessed for symptom development once fungal growth in the control reached the plate edge. The average of the perpendicular diameters of fungal growth was calculated from below the dish and converted to percentage inhibition due to nano material, according to Abbott's equation (1925):

$$\text{Inhibition percentage} = \frac{\text{fungal growth rate in the control} - \text{fungal growth rate in the treatment}}{\text{fungal growth rate in the control}} X\ 100$$

Statistical design

The experiments were carried out according to the completely randomized design (CRD), and the least significant difference (LSD) test at a probability level of 0.05 was used to compare means among treatments. The data were analyzed using the GenStat statistical software.

RESULTS AND DISCUSSION

Effect of the pathogenic fungus R. solani isolated from cucumber on the germination percentages of cucumber seeds in Petri dishes containing Water Agar medium (Water Agar)

The results presented in Table (1) showed a clear, significant variation in the germination percentages of cucumber seeds treated with the pathogenic fungus *R. solani* compared with the control treatment (seeds only). The control treatment recorded the highest germination percentage of 96.0%, which is a normal percentage that reflects the viability of the seeds used and the suitability of the environmental conditions (water agar medium) for germination. Treatment with the fungus *R. solani* led to a clear decrease in germination percentage to 28.5%, reflected in an inhibition percentage of 70.3%. The least significant difference value L.S.D (0.05) of 4.12 indicates that the decrease that occurred is not the result of chance, but rather a direct and strong effect of the pathogenic fungus, as the difference between the control and the treatment (67.5 degrees) far exceeds the L.S.D value, which confirms the high aggressiveness of the tested isolate.

The negative effect of *R. solani* on seed germination is due to complex chemical and biological attack mechanisms. Fungus *R. solani* has a group of cell wall-degrading enzymes. These enzymes break down the structural components of the seed and embryo, leading to tissue damage and degradation before the precursor can erupt above the soil surface or culture medium (Ahmad *et al.,* 2023). Hyphae attack the embryo inside the seed immediately after it absorbs water and initiates vital activity, leading to seed decay. This explains why a large proportion of seeds have not reached the stage of apparent germination (Mondal *et al.,* 2020). In addition to enzymes, the fungus produces secondary metabolites (Toxic metabolites) that cause intoxication of the meristematic tissue in the embryo, stopping cell division and apical growth (Ghoniem *et al.,* 2023).

The antagonistic ability of the fungus T. viridae against the fungus R. solani isolated from cucumber in Petri dishes containing PDA medium

The results in Table 2 showed a significant and clear superiority of the biological fungus T. viride in suppressing and limiting the growth of the pathogenic fungus *R. solani* when both were tested on potato extract medium and dextrose (PDA). This superiority was demonstrated by the recording of a high antagonistic efficiency of 78.4%. This value reflects the high ability of the pathogen to invade the cultural medium and prevent lateral expansion of the fungal colony. Based on Bell›s criterion for evaluating antagonistic capacity, the treatment received a score of (1), the highest degree of biocontrol dominance score, indicating the ability of the resistant fungus to cover the entire surface of the petri dish and grow above the pathogen colony, thus definitively causing the cessation of the latter's growth. The statistical significance measured across the L.S.D (0.05) value of 3.56 reinforces the reliability of these results, as it clearly indicates that the differences recorded between treatments are real and statistically significant, confirming the efficiency of

Table (1) Effect of the pathogenic fungus *R. solani* isolated from cucumber plants on the germination percentages of cucumber seeds in Petri dishes containing water agar medium (Water Agar).

Treatment	Germination (%)	Inhibition relative to the control (%)
Control (seeds only)	95.00	0.00
Pathogenic fungus *R. solani*	27.50	70.40
L.S.D (0.05)	4.14	--

the fungus T. viride as an effective biological control agent.

The success of *T. viride* can be explained in the light of the synergy of several interrelated biological and chemical mechanisms operating in unison, where mycoparasitism emerges as a basic mechanism that begins with the ability of the fungus to molecularly recognize and closely wrap around the filaments of the pathogenic fungus (Nehra *et al.,*2022). This physical engagement is followed by an intense secretion of a group of cellular wall decomposing enzymes, primarily Chitinase and β-1,3-glucanase enzymes, which act on the enzymatic decomposition of chitin and lignin that form the basic structure of the *R. solani* fungal cell wall, resulting in the perforation of fungal hyphae, the loss of their cytoplasmic content, and then their death (Kaur *et al.,* 2021).

In addition to direct enzymatic attack, competition for nutrients and space plays a pivotal role in determining sovereignty in favor of the antagonistic fungus, as the fungus is *T. viride* characterizes the fungus with exceptional growth rates that enable it to deplete the nutrients available in the environmental medium and occupy the spatial space at a speed that exceeds the ability of the pathogenic fungus, putting the latter in a state of food and environmental siege (Manawasinghe *et al.,* 2025). This defense system is complemented by the mechanism of antibiosis, in which the fungus produces a variety of secondary metabolic compounds, whether volatile or non-volatile, that act as strong fungal toxins that inhibit cell division in the pathogen›s hyphae and comprehensively undermine its vital effectiveness.

These results are fully consistent with the findings of recent studies in biological control. Singh et al. (2024) confirmed that modern T. isolates possess superior competitive capabilities, enabling them to secrete antibiotics that inhibit the growth of soil-borne pathogens with high effectiveness. In the local context, the study by Muteab and AL-Abedy (2025) indicated that the genetic diversity of isolates of this fungus in the Iraqi environment confers varying functional properties, making them highly effective in combating soil-borne pathogens, which explains the high efficiency of the isolate used in our study. Al-Abedy et al. (2021) further supported this result by indicating the fundamental role of degrading enzymes in controlling root rot and damping-off diseases. The study by Muhibuddin *et al.* (2021) supports these conclusions by confirming that the interaction between the two fungi always results in significant inhibition of the pathogen, which positively affects plant health. The study by Mahmood and Al-Abedy (2021) also confirms that the efficiency of this biocontrol fungus often surpasses that of conventional chemical pesticides in protecting seeds and seedlings, thereby enhancing the value of the results presented in the present study.

Effect of chelated iron (Fe-EDTA) and chelated manganese (Mn-EDTA) at different concentrations on the growth of the fungus T. viridae and the pathogenic fungus R. solani (cm)

The results presented in Tables 3 and 4 demonstrate a fundamental and significant difference in the response of both the biocontrol fungus *T. viride* and the pathogenic fungus *R. solani* to treatment with the chelated mineral elements, namely iron (Fe-EDTA) and manganese (Mn-EDTA), across different concentrations. Regarding chelated iron, the biocontrol fungus *T. viride* maintained a very high growth rate and remarkable tolerance to elevated concentrations, as its diameter was not severely affected, recording 8.5 cm at the highest concentration (200 PPM) compared to its full diameter in the control treatment In contrast, the pathogenic fungus *R. solani* exhibited a completely different behaviour represented by a sharp and significant decrease in its radial growth to reach 5.2 cm at the same concentration. This pattern of response was repeated when treated with chelated manganese, as the beneficial fungus showed a superior ability to exploit the nutritional medium and grow with an

Table 2. Antagonistic activity of the fungus *T. viridae* against the fungus *R. solani* isolated from cucumber plants in Petri dishes containing PDA medium.

Treatment	Antagonism score (Bell scale)	Inhibition of pathogen growth (%)
T. viridae* against *R. solani	1.4	
Complete dominance of the antagonist		78.40
L.S.D (0.05)	-	3.56

efficiency of 8.8 cm at the highest concentration. In comparison, the pathogenic fungus›s growth decreased to 5.8 cm. The statistical values of the L.S.D confirm that these inhibitory effects were specialized towards the pathogenic fungus, which enhances the hypothesis of the efficiency of the chelating elements in supporting the spatial and biological superiority of the beneficial fungus at the expense of the pathogen.

This variation in response is attributed to fine biological and biochemical mechanisms, where *T. viridae* fungi possess an advanced ability to produce Siderophores, which are organic chelating molecules with a high affinity for capturing iron ions from the surrounding medium and making them available for its growth only. This necessarily deprives the pathogenic fungus *R. solani* of this vital element, which is a basic substrate for its metabolic activities, a phenomenon known as exclusionary competition for nutrients (Abbas *et al.,* 2022). In addition, the biocontrol fungus has highly efficient enzymatic defense systems that enable it to neutralize the potential toxic effects of high mineral concentrations and convert them into factors that stimulate secondary metabolism. These elements act as cofactors that increase the efficiency of degrading enzyme and antibiotic production by the beneficial fungus, whereas pathogenic isolates lack this physiological flexibility, making high concentrations of chelated minerals stressors and growth inhibitors for them (Singh *et al.,* 2024).

These scientific inferences are consistent with findings from recent studies in the integrated management of plant diseases. The study by Sebestyen *et al.,* (2022) indicated that enriching growth media with chelated minerals directly enhances the efficiency of biological control by activating defensive enzymes in beneficial fungi. These results also align with the findings of Muteab and AL-Abedy (2025), who stated that the genetic diversity of T. isolates confers exceptional ability to adapt to mineral stresses in soil and overcome toxicity risks, which supports the present findings regarding the tolerance of the local isolate. In a related context, Al-Abedy *et al.,* (2021) confirmed that enhancing the growth environment of biocontrol fungi with certain inputs can paralyze soil-borne pathogens by activating nutrient-competition mechanisms. On the other hand, the decline in the growth of *R. solani* illustrates what was demonstrated by Hossain *et al.,* (2025) regarding the sensitivity of this fungus to changes in the balance of micronutrients, which leads to deterioration of its pathogenic capacity and vegetative growth, matching the observations recorded in this research regarding the inhibitory role of iron and manganese against strong pathogens.

Effect of nano-chitosan

The experimental results shown in Table 5 indicate a fundamental and significant variation in the response of both the pathogenic fungus *R. solani* and the biocontrol fungus *T. viride* to nano-chitosan treatment

Table 3. Effect of chelated iron (Fe-EDTA) at different concentrations on the colony diameter of *T. viridae* and the pathogenic fungus *R. solani* (cm) after 4 days.

Concentration (ppm)	*R. solani*	*T. viridae*
0 (control)	9.0	9.0
50	7.8	9.0
100	6.7	8.7
200	5.2	8.5
L.S.D (0.05)	0.63	0.22

Table (4): Effect of chelated manganese (Mn-EDTA) at different concentrations on the colony diameter of *T. viridae* and the pathogenic fungus *R. solani* (cm).

Concentration (ppm)	*R. solani*	*T. viridae*
0 (control)	9.0	9.0
50	8.1	9.2
100	7.3	8.7
200	5.8	8.8
L.S.D (0.05)	0.58	0.18

across different concentration levels. The pathogenic fungus showed a sharp and gradual decrease in radial growth with increasing nanomaterial concentration, declining from 9.0 cm in the control treatment to 2.6 cm at 400 mg/L. This decline clearly exceeds the L.S.D. value of 0.43, which demonstrates the high toxicity of nanochitosan towards this pathogen. In contrast to this deterioration in the growth of the pathogen, the beneficial fungus *T. viride* showed a remarkable ability to tolerate and resist, as it maintained a growth rate of 6.7 cm at the highest concentration used, Although a slight decrease occurred compared with the control treatment, its persistence at this activity gives it a very large competitive advantage in colonizing the environment and depriving the pathogen of nutrients and space under nano-stress conditions.

This selective inhibitory effect of nano-chitosan can be attributed to the unique physical and chemical properties of its particles, which are characterized by extremely small size and large surface area, thereby conferring a superior ability to penetrate the cell walls of the pathogenic fungus (El-Gazzar *et al.,* 2023). The positive charges on chitosan play a vital role in electrostatic binding to the negative charges of the fungal cell membrane, leading to changes in membrane permeability and leakage of protein and cytoplasmic contents, ultimately resulting in cell death due to loss of biological balance (Abd El-Wahab *et al.,* 2025). At the same time, nano-chitosan acts as a biological elicitor (Elicitor) that contributes to activating resistance pathways and alerting the defense system, whereas the continued activity of the biocontrol fungus *T. viride* is explained by its possession of defensive enzymatic systems and advanced genetic diversity that enable it to neutralize the potential toxic effects of nano materials or even use them as a carbon and nitrogen source in some cases, which enhances its environmental adaptation capacity and its continuation in performing its antagonistic functions despite the presence of the inhibitory material (Ormeño-Martínez *et al.,* 2024).

These results closely align with recent global research trends toward integrating nanotechnology into biological control systems. Poznanski *et al.,* (2023) confirmed that chitosan nanoparticles act as an antifungal agent via a dual mechanism that includes direct killing of the pathogen and stimulation of beneficial fungal growth. These inferences also align with what Al-Abedy *et al.,* (2021c) stated regarding the superior capacity of nanoparticles to control root rot and damping-off diseases by specifically targeting pathogens and protecting seedlings. In addition, the study by Muteab and AL-Abedy (2025) supports our findings regarding the efficiency of local T. isolates in facing diverse chemical and physical stresses in soil, which explains the tested isolate›s ability to tolerate high concentrations. The sensitivity of the pathogenic fungus *R. solani* recorded in this research is consistent with the study by Zhang *et al.,* (2020), which demonstrated the pathogen›s susceptibility to non-traditional treatments targeting its cell wall and respiratory capacity. In contrast, Zhang *et al.,* (2023) reinforce the idea that improving biological control conditions by adding assisting nano factors increases the ability of the beneficial fungus to suppress pathogens effectively and sustainably, which was actually achieved through the direct pathogen-killing mechanism provided by nano-chitosan in this study.

Effect of the interaction among treatments (best selected concentrations) on the colony diameter of the pathogenic fungus R. solani and the biocontrol fungus T. viridae in Petri dishes (cm)

The results in Tables (6) and (7) indicate a fundamental variation and a high specific response

Table 5. Effect of nano-chitosan (Nano-Chitosan) at different concentrations on the colony diameter of *T. viridae* and the pathogenic fungus *R. solani* (cm).

Concentration (mg/ L)	*R. solani*	*T. viridae*
0 (control)	9.0	9.0
100	6.3	8.6
200	4.2	7.7
400	2.6	6.7
L.S.D (0.05)	0.43	0.32

when the chemical pesticide Tolclofos-methyl was used, whether alone or within the full-interaction treatment. Regarding the effect of the pesticide alone, the data showed a clear superiority against the pathogenic fungus *R. solani*, as its radial growth decreased sharply to 1.9 cm at a concentration of 100 ppm, then achieved complete inhibition (0.0 cm) at the higher concentrations (500 and 1000 ppm). In contrast, the biocontrol fungus T. viride showed notable tolerance to this pesticide, maintaining a growth rate of 7.1 cm at 100 ppm, and continued to exhibit biological activity even at the highest pesticide concentration, recording 3.2 cm. When the full-interaction results in Table (7) were examined, the maximum applied value of the research became evident, as the combined treatment that included the biocontrol fungus, nano-chitosan, micronutrients, and half of the recommended pesticide dose led to complete inhibition of the pathogen (0.0 cm), while ensuring the continued growth of the beneficial fungus at a good rate of 6.1 cm. This result is significantly superior to the efficacy of each component of the interaction when applied alone, providing statistical and biological evidence of synergy among these factors.

The qualitative success of these interactions is attributed to the integration of killing and stimulation mechanisms across different chemical and biological pathways. Tolclofos-methyl acts by directly targeting the construction of phospholipids in the fungal cell membrane of the pathogen, whereas strains of the beneficial fungus T. possess advanced defensive and physiological systems, such as efflux pumps, which enable them to expel chemical toxins or enzymatically degrade pesticide molecules, explaining their continued activity in a medium containing the pesticide (Oliver *et al.,* 2022). In the context of biological-chemical synergy, the presence of nano-chitosan disrupts the cell wall structure of pathogenic cells and alters their permeability, facilitating the penetration of pesticide molecules and micronutrient ions (iron and manganese) into the cytoplasm. These elements then act as co-factors that increase the efficiency of lytic enzyme and fungicidal toxin production in the beneficial fungus, rendering the pathogenic fungus easy prey for the combined attack of the biocontrol agent (Hernández-López *et al.,* 2025).

These results are in line with the leading scientific directions in the field of integrated pest management (IPM), where the research of Kumar *et al.,* (2024) confirmed that the integration of low doses of pesticides with biocontrol agents represents an effective strategy to reduce the risk of the emergence of pesticide-resistant strains while maintaining the ecological balance within the soil. The interpretation of the genetic flexibility of the beneficial fungus also aligns with what Muteab and AL-Abedy (2025) stated regarding the presence of local T. isolates that are exceptionally efficient in confronting chemical and physical stresses and in decomposing complex compounds. These inferences are supported by the study by Al-Abedy *et al.,* (2021c), which indicated that supporting biocontrol fungi with nano- or mineral-based inputs enhances their competitive and antagonistic abilities by activating their intrinsic defensive systems. Accordingly, adopting a multi-pathway attack strategy, as embodied in this study, ensures suppression of the pathogen through mechanical degradation, chemical toxicity, and nutrient competition at the same time, which is consistent with the view of Kumar *et al.* (2024) regarding the necessity of diversifying selective pressures on strong pathogens to prevent the development of their defensive capacities and to ensure sustainable protection of crops.

Table 6. Effect of the pesticide tolclofos-methyl 50% at different concentrations on the pathogenic fungus *R. solani* and the biocontrol fungus *T. viridae* (cm).

Concentration (mg L-1)	*R. solani*	*T. viridae*
0 Control	9.0	9.0
100	1.9	7.1
500	0.0	5.6
1000	0.0	3.2
L.S.D (0.05)	0.26	0.49

Table 7. Effect of the interaction between treatments (best selected concentrations) on the colony diameter of the pathogenic fungus *R. solani* and the biocontrol fungus *T. viridae* in Petri dishes (cm).

Interaction treatment	*R. solani*	*T. viridae*
Control (without additives)	9.0	9.0
T. viridae + iron + manganese	4.3	8.9
T. viridae + nano-chitosan	2.2	7.8
T. viridae + chitosan + elements + pesticide (half dose)	0.0	6.1
L.S.D (0.05)	0.34	0.28

Effect of the interaction among treatments on infection percentages of damping-off disease caused by the fungus R. solani and some growth parameters of cucumber plants in plastic pots

This study culminated in the results of the pot experiments, which served as the true and realistic standard for evaluating the efficiency of the tested treatments under conditions simulating the complex conditions of the soil. The data presented in Table 8 revealed a fundamental and decisive variation in the levels of plant protection and growth enhancement. The treatment with the pathogenic fungus *R. solani* alone showed high aggressiveness which led to heavy losses, represented in the death rate of seedlings before emergence to 46.0% and after emergence to 33.5%, %, which caused the percentage of surviving plants to fall to 22.4% only with a weak shoot dry weight that did not exceed 0.44 g, which confirms the high destructive capacity of this pathogen in the absence of means of protection. On the other hand, full-interaction treatment, which combined the biocontrol fungus, nano-chitosan, and micronutrients with half the pesticide dose, recorded a very large and decisive superiority, achieving the highest plant survival percentage of 95.6%, with the overall damping-off percentage decreasing to its lowest level (3.0%). In addition, it recorded a qualitative jump in shoot dry weight reaching 1.93 g, an increase of about 332% compared with the pathogen-only treatment. The statistical significance measured by the L.S.D. values for all studied parameters confirms that this superiority was not a matter of chance, but rather the result of a synergistic formulation that achieved a large, statistically significant difference, reflecting the successful translation of laboratory results into field applications.

This notable superiority of the full-interaction treatment is attributed to the adoption of a "multi-axis defensive strategy" that integrates preventive and curative protection and growth stimulation simultaneously. Nano-chitosan and the reduced-dose chemical pesticide acted as a first line of defence that provided direct inhibition of the pathogen and destabilization of its cell walls, which paved the way for the biocontrol fungus *T. viride* to colonize the rhizosphere zone and attack the remaining pathogen hyphae and destroy them through a specialized mycoparasitism mechanism (Elsharkawy *et al.,* 2024). The benefits of this formulation are not limited to suppressing the causal agent only, but extend to include the role of the biocontrol fungus and the mineral elements (iron and manganese) as biological growth stimulants (Bio-stimulants) that increased nutrient availability for the plant and acted as cofactors for producing defensive enzymes such as peroxidase and PPO within cucumber tissues (Singh *et al.,* 2022). In addition, nano-chitosan contributed to activating induced systemic resistance (ISR), making the seedlings more resilient to any late fungal attacks, while the use of the local isolate of T. ensured sustainability of biological activity and high adaptive capacity to local soil conditions throughout the crop growth period (Khafaji *et al.,* 2024).

These results and analyses closely align with leading scientific trends calling for the adoption of sustainable agriculture models. Gautam *et al.* (2025) stated that technical formulations integrating nano, biological, and reduced-chemical components provide dual protection and enhance plant biomass to an extent that exceeds that achieved with each factor alone. This view is consistent with what Al-Abedy *et al.* (2021c) confirmed regarding the role of integration between nano and biological components in destroying pathogens through multiple mechanisms, including enzymatic degradation and immune stimulation. A study by Sen *et al.* (2025) also supports

Table 8. Effect of the interaction between treatments on damping-off incidence caused by the fungus R. solani and growth parameters of cucumber plants in plastic pots.

Treatment (in pathogen-infested soil)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Surviving plants (%)	Shoot dry weight (g plant-1)
Pathogen only (*R. solani*)	46.0	33.5	22.4	0.44
Pathogen + *T. viridae*	16.0	11.0	77.0	1.15
Pathogen + *T. viridae* + micronutrients	13.0	9.0	81.0	1.48
Pathogen + nano-chitosan + *T. viridae*	7.0	6.0	89.0	1.67
Combined treatment (all treatments)	2.6	3.0	95.6	1.93
L.S.D (0.05)	**4.7**	**3.9**	**5.6**	**0.16**

the findings related to the role of nano-chitosan as a resistance elicitor and promoter of vegetative growth, while AL-Abedy and Muteab (2025) explain the sustained success of this treatment in the field by the high capacity of local Trichoderma isolates for environmental adaptation and resistance to chemical stresses. Thus, this study forms a logical sequence that began with diagnosing the pathogen›s virulence and ended with developing a precise synergistic combination that achieves the highest standards of protection and productivity with the least possible environmental impact, making it an ideal model for safe and sustainable agricultural applications.

CONCLUSION

The study concludes that the application of a multi-track attack strategy based on the synergy among bioresistance (*T. viride*), nanotechnologies (Nano-Chitosan), and chelated metal elements represents an effective and sustainable model for the management of cucumber seedling damping-off. This integration demonstrated superior ability to neutralize the pathogenic fungus *R. solani* and increase seedling survival to 95.6%, while achieving a 333% increase in plant growth. Most importantly, this strategy allowed for a 50% reduction in reliance on traditional chemical pesticides, ensuring high environmental protection without compromising control efficiency or crop quality.

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ORIGINALITY AND PLAGIARISM:

The author confirms that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All sources used have been appropriately cited, and the manuscript has been prepared in compliance with plagiarism and publication ethics standards.

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The author solely conceived and designed the study, conducted the experiments, collected and analyzed the data, and wrote and revised the manuscript.

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