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ARTICLE

Synthesis of Zinc Oxide Nanoparticles using *Trichoderma* sp. and testing their efficacy in inhibiting the damping-off disease on three plants of the Solanaceae

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ABSTRACT

The study aims to provide environmentally friendly and safe alternatives to traditional chemical pesticides by preparing zinc oxide nanoparticles (ZnO NPs) using the filtrate of *Trichoderma* sp. The inhibitory efficacy of nanoparticles against the pathogenic fungus Fusarium sp. was evaluated *in vitro*, and their ability to reduce the severity of damping-off disease was assessed in three plant species (Tomato, Pepper, and Eggplant). The synthesis of the ZnO NPs was confirmed using a UV spectrophotometer, with peak absorption at 342 nm. The results showed a significant decrease in the growth of the phytopathogenic *F. proliferatum* with increasing concentration, reaching 90.45% inhibition at 1000 ppm, compared to the control treatment where the fungus grew to full size (9 cm). The use of the nanoparticles at a concentration of 1000 ppm significantly reduced the incidence of damping-off compared to the control treatment alone. The mortality rate in eggplant plants decreased to 10% (down from 40% when infected solely with the pathogen), in tomatoes to 15% (down from 30%), and in peppers to 40% (down from 70%). The use of the *Trichoderma* in the production of ZnO NPs is beneficial due to its dual effectiveness and its contribution to sustainable agriculture, aiming to reduce the harmful environmental impacts of chemical pesticides.

Keywords: *Mycosynthesis; ZnO-NPs; Inhibition Rate; Damping-off*

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INTRODUCTION

Tomatoes (*Solanum lycopersicum L.*), chili peppers (*Capsicum annuum* L.), and eggplants (*Solanum melongena* L.) are major crops belonging to the nightshade family, native to subtropical and tropical regions. Tomatoes, peppers, and eggplants are rich sources of vitamins and minerals essential for human health. They also contain a variety of phytochemicals, such as polyphenols, which are bioactive compounds with numerous health benefits, including antioxidant, anti-inflammatory, anticancer, and lipid-lowering properties (Padmanabhan *et al*., 2016). Cultivation of these plants is widespread in most Iraqi governorates, particularly in Karbala and Babylon, as well as in the central and southern regions where sunny weather prevails. Recently, greenhouse cultivation has also been developed in Iraq to ensure year-round availability, thanks to the suitable conditions provided inside the greenhouses. Despite Iraq's vast agricultural lands, its productivity is relatively low compared to other countries. Studies have indicated that the high rate of fungal and viral diseases is one of the reasons for the decline in its productivity (Panno *et al*., 2021). Soil-borne fungi are among the most important pathogens that cause seedling damping-off disease, such as *Rhizoctonia* sp. *Fusarium* sp. and Oomycetes parasites such as *Pythium* sp. and *Phytophthora* sp. which causes the death of more than 60% of seedlings in both nurseries and fields (Manoranjitham and Prakasam, 2000;

Jadhav and Ambadkar, 2007).

Damping-off diseases are among the most serious diseases affecting crops, including those in the Solanaceae family, as they reduce productivity and cause deterioration in crop quality. Soil-borne fungi are significant plant pathogens that are difficult to control using traditional methods, necessitating the search for safe and effective alternatives (Delai *et al*., 2024). Green nanotechnology is one of the most prominent modern scientific trends that aim to produce nanomaterials in environmentally friendly and inexpensive ways, moving away from traditional chemical and physical methods that may have toxic side effects (Singaravelu *et al*., 2025). In recent years, nanotechnology has gained widespread attention in agriculture due to its potential to improve biological control efficiency and reduce environmental damage. Zinc oxide nanoparticles (ZnO NPs) stand out as one of the most important metal oxides due to their unique physical and chemical properties and their high capacity as an antimicrobial agent (Nandhini *et al*., 2024). Some of the *Trichoderma* sp. fungi are important microorganisms in biological control due to their ability to inhibit plant pathogens and produce effective compounds (Tomah *et al*., 2020). Recent studies have focused on utilizing *Trichoderma* in the biosynthesis of nanoparticles in an environmentally friendly and safer manner compared to chemical methods. Controlling eggplant diseases using nano-*Trichoderma* represents a smart integration of biological control and modern technology. The use of *Trichoderma* sp. in nanoparticle biosynthesis (mycosynthesis) is a highly effective method due to these fungi's ability to secrete large quantities of enzymes and proteins that act as natural reducing agents and capping agents (Tomah *et al*., 2023). Solanaceae crops face significant production challenges due to damping-off disease caused by soil-borne fungal pathogens, leading to substantial economic losses. This study explores the potential of integrating bio-nanotechnology and biocontrol to find safe and effective alternatives to chemical pesticides. The research aims to biosynthesize zinc oxide particles using the fungal filtrate of *Trichoderma* and evaluate their efficacy against *Fusarium* sp. growth *in vitro*. It also examines their ability to reduce the severity of damping-off disease caused by this pathogen in three plant species belonging to the Solanaceae family.

MATERIALS AND METHODS

Preparation of Fusarium and Trichoderma Isolates

The pathogenic fungus *Fusarium proliferatum* and three *Trichoderma* isolates (BY1, HZA84, and HZA14) were obtained from the Plant Pathology Laboratory, College of Agriculture- University of Maysan. The isolates were re-cultured on potato Dextrose Agar (PDA) at 25 °C in an incubator for 7 days and then refrigerated until use. To prepare the Trichoderma isolate filtrates, five 0.5 cm mycelial discs of each isolate were transferred to 250 ml flasks containing 100 ml of PD broth and incubated at 25 °C with continuous stirring. After 14 days of incubation, the fungal colonies were separated from the culture filtrate by filtering through filter paper, and then centrifuged at 3000 rpm for 10 minutes, and finally purified using a 0.22 µL Millipore membrane. The filtrate was stored in the refrigerator until use (Tomah *et al*., 2020).

Preparation of Zinc Nitrate and the Fungicide Avistin FL

White zinc nitrate with 98% purity was purchased in a 150-gram packet (manufactured by Hunan Heaven Material Development Co., Ltd., China). The fungicide Avistin FL was purchased from an agricultural office in Maysan Governorate. The fungicide Avistin FL was purchased from an agricultural office in Maysan Governorate. The active ingredient is Carbendazim 500g/LC. The recommended application rate is 10 grams per 20 liters of water. Therefore, 0.5 grams were diluted to 1 liter of water.

Synthesizing Zinc Nanoparticles (ZnO NPs)

ZnO NPs were synthesized biosynthetically using three *Trichoderma* isolates, following the method described by Tomah *et al*. (2022). To prepare the *Trichoderma* filtrate, 10 fungal discs of each *Trichoderma* isolate were placed in a 250 mL flask containing 100 mL of sterile PD broth medium and incubated at 25 °C for 7 days. The biomass (mycelium) of the *Trichoderma* isolates was collected, washed thoroughly twice with deionized water, and dried on filter paper. Ten grams of biomass were weighed per isolate and transferred to 250 ml flasks containing 100 ml of deionized water. The flasks were incubated at 25 °C for 3 days. The filtrate for each isolate was obtained after mycelial removal by filtering it through Whatman No. 1 filter paper and 0.45 μL Millipore membrane filters. The filtrate for each sample was mixed with a 1 mM zinc nitrate solution, prepared using zinc nitrate dissolved in distilled water.

The reaction mixtures were incubated in a rotary shaker at 30°C for 4 hours to complete the reaction. Once the reaction was complete, the components were separated using a centrifuge. The product was placed in tubes in the centrifuge for 15 minutes at 300 rpm. The liquid was then discarded, and the sediment at the bottom of the tubes, formed from the separation process, was collected. This sediment was then dried in a Petri dish at 60 °C for 4 hours to ensure the sample was completely dry. Finally, the sample was ground in a ceramic mortar and placed in a special container to prevent oxidation from sunlight.

Characterization of ZnO NPs Using Spectroscopy

The prepared ZnO NPs were characterized and stabilized by dissolving 0.1 g of the residual zinc oxide nanoparticles in 10 mL of dimethyl sulfoxide (DMSO). The solution was then subjected to ultrasound for 15 minutes and analyzed using ultraviolet spectroscopy. Both optical and absorbance measurements were recorded. The instrument was zeroed using the dissolving agent, and the sample was then analyzed. The spectroscopic readings of the ZnO NPs ranged from 200 nm to 800 nm (Alamdari *et al*., 2020; Khan *et al*., 2023).

Impact of ZnO NPs on *Fusarium proliferatum in Vitro*

A quantity of ZnO NPs was mixed with molten PDA medium before solidification to obtain concentrations of 250, 500, and 1000 ppm. 10 mL of each concentration was poured into sterile 9 cm plastic Petri dishes. After the medium solidified, 0.5 cm mycelium discs (made using a sterile cork piercer) were transferred from the *F. proliferatum* colony to the center of Petri dishes containing the ZnO NPs concentrations. Discs of the same size were transferred to Petri dishes containing PDA medium without ZnO NPs as a control. The dishes were incubated at 25°C. The size of the *F. proliferatum* colonies at each concentration was measured using a ruler by two perpendicular diameters. The inhibition rate of the pathogenic fungus for each concentration used was calculated using the following equation: Inhibition % = (Pathogenic colony diameter in control treatment - Pathogenic colony diameter in ZnO NPs treatment) / Pathogenic colony volume in control treatment × 100.

Impact of ZnO NPs Produced on Damp-Off Disease in Seedling Trays

A quantity of peat moss was mixed with sandy soil in a 1:1 ratio. The mixture soil was autoclaved twice at 121°C and 15 psi for 30 minutes. The autoclaved soil was distributed in plastic seedling trays. The tray soil was inoculated with 0.5 mL of a 2 x $^{6}10$ concentration of the pathogenic fungus conidia, predetermined using a hemocytometer slide. Eggplant, tomato, and pepper seeds were sown in the seedling trays and immediately watered with 1000 ppm ZnO-NPs, the recommended concentration of Avistin FL fungicide, and sterile distilled water as a control treatment, according to the following treatments:

1-Treatments involving only the *F. proliferatum*

2- Treatments involving the *F. proliferatum* and the fungicide

3-Treatments involving the *F. proliferatum* and 1000 ppm ZnO NPs

4- Treatments involving sterile soil without the *F. proliferatum*

The trial trays were transferred to a greenhouse, and the growth conditions were set at 25°C, and relative humidity of 70-80%. The 21 days post-planting, the percentage of damping-off was calculated according to the equation: damping-off %= [(Number of damping-off seedlings / Total of germinated seedlings)] × 100.

Statistical Analysis

All trial units were implemented with 4-replicates. Excel was used to extract the confidence (±) among the replicates of the means. SAS software version (9.1.3) was used to analyze the data with a completely randomized design (CRD). A 5% significance level was used in the least significant difference (LSD) test among the means.

RESULTS AND DISCUSSION

Synthesis of Zinc Oxide Nanoparticles

Of the three *Trichoderma* isolates tested, the filtrate of isolate HZA14 successfully synthesized zinc oxide nanoparticles, forming a gel-like suspension that precipitated as a white powder (Figure 1A and B). The synthesis of metallic nanoparticles using *Trichoderma* isolate filtrate is a green chemistry approach, where the fungal metabolites act as reducing and stabilizing agents (Tomah *et al*., 2022). This biomethod is environmentally friendly as it does not require toxic chemicals. *Trichoderma* fungus produces metabolites and enzymes that reduce zinc ions (Zn^{2+}) to zinc oxide nanoparticles (Consolo *et al*., 2020; Mishra *et al*., 2025).

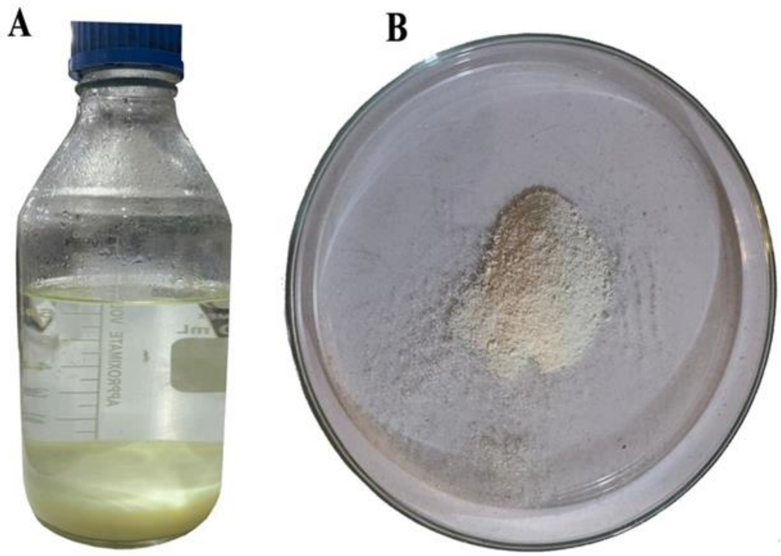

Figure (1) Biosynthesis of Zinc nanoparticles (ZnO-NPs) mediated by the culture filtrate of *Trichoderma* isolate HZA14. (A) ZnO-NPs suspension post biosynthesis. (B) ZnO-NPs powder product post-grinding.

Preliminary Examination of Produced ZnO-NPs

The formation of zinc oxide nanoparticles using the *Trichoderma* sp. culture filtrate was investigated and confirmed by UV spectrophotometry (Figure 2). The spectrophotometry showed an absorption peak at 342 nm, indicating the biosynthesis of zinc oxide nanoparticles using the *Trichoderma* HZA14 isolate filtrate. These results are consistent with the study published by the researcher (Sumanth *et al*., 2020; Zaki *et al*., 2021).

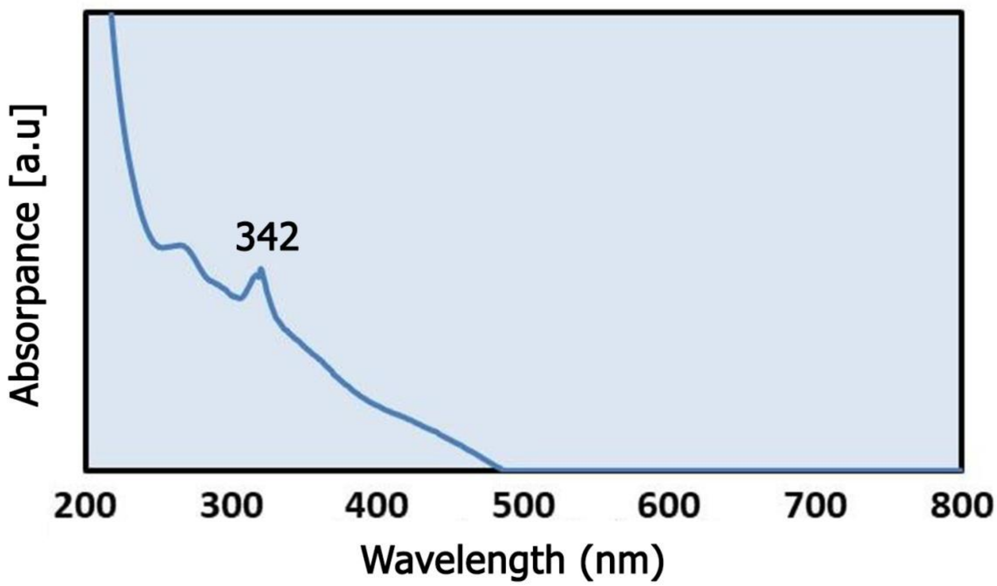

Figure (2) UV- visible spectra of the ZnO-NPs mediated by the culture filtrate of the isolate HZA14

Impact of ZnO NPs on Inhibiting *Fusarium proliferatum* Growth

The potential of ZnO-NPs as an antifungal agent was investigated by culturing *F. proliferatum* in PDA supplemented with various concentrations of ZnO-NPs. The results showed that ZnO NPs significantly reduced pathogenic fungal growth in the cultures at concentrations of 250, 500, and 1000 ppm, reaching 6.47, 2.89, and 0.86 cm, respectively, compared to the control, where the average colony growth rate of the pathogenic fungi was 9 cm (Figure 3). The results also demonstrated that ZnO-NPs inhibited *F. proliferatum* growth at the aforementioned concentrations by 28.11%, 67.88%, and 90.45%, respectively (Table 1). The results show that the highest concentration, 1000 nanoparticles of ZnO-NPs, inhibited colony growth by 90%, while a concentration of 500 nanoparticles inhibited colony growth by 67%. In contrast, the control treatment containing only the pathogen allowed for complete growth. This is attributed to the fact that zinc nanoparticles act in several ways, including oxidative stress through binding to the fungal cell wall, disrupting the cell wall and membrane, and damaging proteins and DNA. Furthermore, the Trichoderma used in particle synthesis contains substances and toxins that impair the fungus's ability to grow and produce reproductive parts. The zinc ion (Zn^{2+}) released into the environment affects the ionic balance within the fungal cell and inhibits the enzymes responsible for fungal metabolism and respiration. It also inhibits growth enzymes and distorts the fungal mycelium. Additionally, the binding of *Trichoderma* particles to the nanoparticles increases their efficiency in penetrating and binding to the fungal cell wall (Zaki *et al*., 2012).

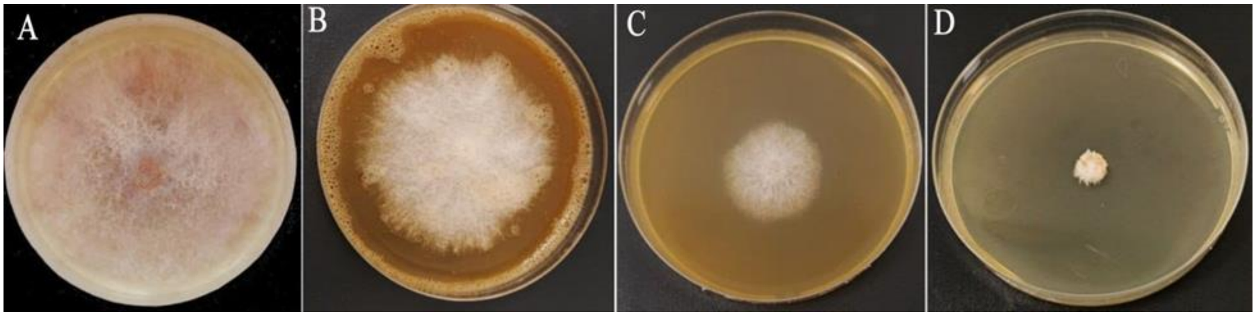

Figure (3) Antifungal activities of ZnO-NPs biosynthesized by isolate HZA14 against of *F. proliferatum* in PDA. (A) Treatment with 0 ppm of ZnO-NPs. (B) Treatment with 250 ppm of ZnO-NPs. (C) Treatment with 500 ppm of ZnO-NPs. (D) Treatment with 1000 ppm of ZnO-NPs.

Table (1) Antifungal the different concentrations of ZnO-NPs biosynthesized by isolate HZA14 against the growth of *F. proliferatum* in PDA

Treatments	Radial Growth/ cm	Inhibition %
ZnO-NPs 250 ppm	6.47	28.11
ZnO-NPs 500 ppm	2.89	67.88
ZnO-NPs 1000 ppm	0.86	90.45
Control	9.00	——

Impact of ZnO NPs on Controlling Damp-Off Disease Caused by *F. proliferatum*

The results showed that adding zinc oxide nanoparticles at a concentration of 1000 ppm reduced the incidence of damp-off disease caused by the pathogenic fungus *F. proliferatum* in tomato, pepper, and eggplant plants. Treating the soil with ZnO-NPs via irrigation in seedling trays resulted in greater protection for the seeds and seedlings of the cultivated plants. The damping-off rate was 40% in tomatoes, 30% in eggplant, and 70% in peppers, while the damp-off rate in the pathogen-treated plants was 80-90% (Table 2).

The results was consistent with the findings of Zaki *et al*. (2021) on the potential to reduce the damp-off rate of cotton seedlings caused by *Fusarium* sp., *Rhizoctonia solani*, and *Macrophomina phaseolina* when seeds are soaked in ZnO-NPs. Furthermore, zinc oxide nanoparticles synthesized using the extracellular filtrate of *Trichoderma asperellum* exhibit potent antifungal activity through a combination of physical destruction and biochemical interference against the pathogenic fungus *Fusarium equiseti* (El-Morsy *et al*., 2026).

Table (2) The impact of ZnO-NPs biosynthesized by isolate HZA14 at 1000 ppm on reducing damping-off caused by phytopathogenic *Fusarium proliferatum* in eggplant, tomato, and pepper seedlings in greenhouse conditions

Treatments	Damping-off%		
	eggplant	Tomato	pepper
Pathogen only	40	30	70
Avistin FL fungicide	45	25	50
ZnO-NPs 1000 ppm	10	15	40
Control	0.0	0.0	0.0

CONCLUSIONS

The extract of *Trichoderma* isolate HZA14 can produce zinc oxide nanoparticles that are more stable and have a longer-lasting effect than the fungicide used in the laboratory to inhibit pathogenic fungi *F. proliferatum*. The efficiency of nanoparticles has been proven in suppressing the development of seedling drop disease on plants of the Solanaceae family. The trend towards using pesticides made from nanoparticles is an environmentally friendly method, in addition to its introduction into modern sustainable agriculture and moving the world towards a sustainable environment and less use of chemical pesticides that harm the ecosystem and leave harmful residues in the environment.

Conflict of interest: The authors declare that they have no relevant financial or non-financial interest to disclose.

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