

ARTICLE

Evaluation of the Inhibitory Efficacy of a Green-synthesized ZnO Nanoparticle Prepared Using *Beta vulgaris* L. against Some Root Rot Fungi in the Orchards of Karbala Governorate

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ABSTRACT

Root rot diseases are significant problems affecting many plants due to infection by various pathogenic fungi, necessitating the search for effective and environmentally safe control methods, such as the use of green-synthesized zinc oxide nanoparticles (ZnO-NPs). The aim of this study is to evaluate the effectiveness of green-synthesized zinc oxide nanoparticles in inhibiting fungi that cause root rot in certain agricultural plants. *Beta vulgaris* L. has been used for this purpose, as it is rich in compounds with antimicrobial properties. The plant has attracted increasing consideration for its possible role in combating many plant pathogens, particularly root diseases, which are among the most significant factors affecting many plant hosts, including *C. roseus*, *S. lycopersicum* and *G. hirsutum*, and pose a major threat to these plants. Samples were collected from the roots of certain plants that have root rot (*S. lycopersicum*, *C. roseus*, *G. hirsutum*) from different orchards in Karbala Governorate. Morphological and microscopic traits were used to identify the isolated fungi. The following species were presumptively identified: *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium solani*. They were treated with nano-zinc oxide prepared from the water-based extract of the *B. vulgaris* L. and zinc acetate, followed by heating and stirring, then drying and calcination. Subsequently, various concentrations of ZnO nanoparticles were added to the PDA medium containing the fungi; the plates were incubated, and fungal growth was measured to determine the inhibition rate. The findings demonstrated a clear correlation between the extract's ZnO concentration and its ability to suppress the fungus that was isolated from the diseased plants' roots. The concentration of 200 ppm recorded the highest rate of inhibition diameter compared to the other concentrations used. The inhibition rates were 85.56%, 83.33%, and 80.00% for *A. alternata*, *F. solani*, and *R. solani*, respectively, with a clear difference in the degree of response between the fungal species. Zinc oxide nanoparticles made from *B. vulgaris* L. aqueous extract exhibited inhibitory activity against the isolated fungi, confirming their antifungal efficacy.

Keywords: Green Nanotechnology; *Beta vulgaris* L.; Root Rot Disease; Inhibitory Activity

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INTRODUCTION

Nanotechnology is a field aimed at developing and producing materials that range in size from one to one hundred nanometers (Zeebaree *et al.*, 2022), which leads to significant changes in physical and chemical properties compared to larger materials, resulting in advanced materials with wide applications (Basheer, 2020). Green synthesis is one of the most important applications of modern nanotechnology (Adim and Abbas, 2023). It is characterized by its low cost and environmental friendliness, as it uses biologically derived reducing agents. The organisms participating in the synthesis interact with various metal salts, reducing them to nanoscale materials. After appropriate characterization, these materials can

be used for many different purposes (Bedida and Choughrem, 2023). For instance, Zinc oxide nanoparticles (ZnO-NPs) have unique physical characteristics that make them one of the most significant families of metal oxide nanoparticles, including size, shape, and crystal structure, as well as their economic viability and biocompatibility. Zinc oxide nanoparticles (ZnO NPs) possess antifungal properties; they can affect fungal cells by damaging the cell wall and membrane and increasing oxidative stress, thereby inhibiting fungal growth. ZnO nanoparticles are synthesized using various methods, including “green synthesis,” which relies on plant extracts as biological agents for particle formation and stabilization. The sugar beet plant (*Beta vulgaris*) is one of the plants used for this purpose. *Beta vulgaris* L. is a root vegetable characterized by its spherical or conical shape and vibrant color. It belongs to the Amaranthaceae family (Edziri et al., 2020). It is characterized by being rich in water-soluble nitrogen pigments known as betalains, which have long been used as natural food colorings (Kamli et al., 2021). Numerous studies have shown that the biochemical analysis of *B. vulgaris* L. can include its water, carbohydrate, protein, fiber, fat, and various essential mineral content (Sharma et al., 2023). It is also rich in polyphenols and betalains, both of which possess antimicrobial properties and act as antioxidants, combating oxidative stress known to increase susceptibility to microbial infections (Bouchmaa et al., 2022). These bioactive compounds make *Beta vulgaris* a promising plant for the green synthesis of ZnO-NPs, which have demonstrated potential antifungal activity against various plant pathogens, including those associated with root rot diseases. Root rot diseases are among the most important diseases that threaten many plants, as they cause the roots to turn brown and their tissues to decompose, leading to yellowing and wilting of the leaves, cessation of plant growth, reduced yield, and crop loss (Han et al., 2025). The causes of root rot are attributed to various groups of microbes like fungal and bacterial species; among the important fungal pathogens associated with root rot are *Alternaria alternata*, *Rhizoctonia solani*, and *Fusarium solani*. Insects and nematodes also actively contribute to germs and fungus entering the roots by causing wounds and scratches to the plant, and leads to infection (Legg et al., 2011).

As a perennial ornamental plant, *Catharanthus roseus* L. is classified within Apocynaceae, and is widely cultivated in Iraq (Jaleel and Panneerselvam, 2008). This plant is distinguished by its attractive appearance and vibrant, varied flower colors. It is also highly valued for its medicinal properties, containing a number of active phytochemicals. The *Solanum lycopersicum* is one of the most famous vegetables grown worldwide, due to its high nutritional and economic value. It is a significant source of vitamin C, lycopene, and antioxidants, which contribute effectively to promoting human health (Balali et al., 2025). *Gossypium hirsutum* is one of the utmost vital yields trending the world, cultivated in more than 80 countries for the manufacture of typical fibers used in the fabric manufacturing (Vitale et al., 2025). Its seeds are also used in the production of oils and animal feed. *Gossypium hirsutum* holds significant economic and agricultural importance, and many recent studies have focused on it due to its richness of bioactive chemicals. These plants are susceptible to infection that impact their growth and productivity. Among the most important root rot fungi are *Pythium aphanidermatum*, *S. sclerotiorum*, *R. solani*, and *F. solani*. Other fungi that infect the foliage include *Phytophthora* and *A. alternata* (Ibanes et al., 2022).

These plants are widely cultivated in Karbala Governorate and, like other crops, are vulnerable to various pathogens. Therefore, controlling these pathogens is crucial for maintaining plant health and quality. The study offers empirical support for the possible application of a *B. vulgaris* L. nano-extract as an antifungal agent against plant pathogens, especially those that are linked to root rot diseases. Its importance stems from investigating a natural plant source and a nanotechnology-based strategy that may help create more sustainable substitutes and lessen dependency on traditional chemical fungicides. Future research could be expanded upon in future studies to clarify the antifungal mode of action, improve the nano-extract's formulation and concentration, and assess its effectiveness in real-world settings.

MATERIALS AND METHODS

2.1 Specimens Collection

The specimens were taken from the roots of three different plant species: *Catharanthus roseus*, *Gossypium hirsutum*, and *Solanum lycopersicum*. The distribution of the fungal isolates among the plant hosts was as follows: *Alternaria alternata* and *Fusarium solani* were isolated from *Solanum lycopersicum*, whereas *Rhizoctonia solani* was recovered from *Gossypium hirsutum* and *Catharanthus roseus* from various orchards of Al-Husayniyah city in Karbala Governorate during the period of May to June 2026. The specimens were collected based on visible signs such as yellowing leaves and wilt. The infected root specimens were collected, placed in sterile bags, and transported directly to the postgraduate laboratory at the University of Kerbala for analysis and fungal isolation.

Isolation of Fungi Associated with Root Rot

The infected roots were cleaned of dirt and debris using running water, and were then cut into tiny pieces of 0.5-1 cm at the boundary between healthy and infected tissue. The pieces were surface-sterilized with a 1-2% sodium hypochlorite solution for two min., then washed three times with distilled water to remove any residue of the sterilizing agent, and were finally dried on sterile filter paper. Sterile plant sections were cultivated on potato dextrose agar (PDA) medium—prepared according to the instructions of the Italian manufacturer Liofilchem by dissolving 42 g in 1,000 mL of sterile distilled water and pouring the mixture into Petri dishes (9cm) and the dishes were incubated at 25 ± 2 °C for 5-7 days, with daily monitoring of fungal colony growth. After fungal growth appeared, the tips of developing hyphae or single spores were transferred to new dishes containing PDA medium to obtain pure isolates, and then incubated again under the same conditions until contamination-free colonies were obtained.

Identification of Isolated Fungi

The isolated fungi were identified based on their morphological and microscopic features, including the shape, color, and texture of the colonies, as well as their growing rate. Microscopic slides of the purified isolates were prepared and examined under a light microscope to observe the diagnostic features of sporangia, spores, and other reproductive parts. Diagnostic features corresponded with established taxonomic keys to identify the genera and species of the isolated fungi (Watanabe, 2010; Bradley and Ajayi-Oyetunde, 2018).

Green Synthesis of Zinc Oxide Nanoparticles Using *B. vulgaris* L. Aqueous Extract

The method of Al-Nuaimi (2026) was followed for the green synthesis and the experiment included the following steps:

Preparation of the Extract

The common beet *B. vulgaris* L. roots were gathered, properly cleaned with water to eliminate dirt, dried at the proper temperature, and grounded into a powder. An aqueous extract was then made using this powder. A 5 g of *B. vulgaris* root powder was dissolved in 200 ml of distilled water and heated in a water bath at 50-60 °C. For half an hour, the mixture was filtered through medical gauze. A centrifuge at 3000 rpm and ordinary filter paper were then used to completely separate the sediment. The extract was dried at room temperature (25 °C) for 3-5 days and the dry extract was collected. The required concentration for the green synthesis was prepared by melting 1g of the dry plant extract in 10 ml of deionized water (considered the stock solution). 750 µL of this solution was added to 150 ml of desalinated water and mixed thoroughly. 25 ml of this mixture was then used to synthesize the nanomaterial. The

resulting concentration is the lowest concentration of the aqueous extract of the *B. vulgaris* L., 0.5 mg/ml, which is required in the green synthesis experiment (Borjan *et al.*, 2022).

Preparation of a Zinc Acetate Solution

To prepare a zinc acetate [$\text{Zn}(\text{CH}_3\text{COO})_2$] solution at 0.02 M (20 mmol), 3.67 g of zinc acetate hydrate was dissolved in 800 mL of deionized water with continuous shaking, and then the volume was increased to 1000 mL. Its country of origin is the USA while Difco is the manufacturer.

Manufacturing Experiment

25 ml of aqueous *B. vulgaris* L. extract was gradually added to 500 ml of 0.02 M zinc acetate solution by slow distillation, maintaining the temperature at 65°C with continuous stirring using a magnetic stirrer to prevent clumping or irregular precipitation. The pH was measured after the addition of the plant extract, and then a few drops from NaOH solution were added gradually with gentle stirring until the desired value (12) was reached. After half an hour, the heat was turned off, and the mixture was left to mix for one hour to ensure complete reaction. A cloudy, milky mixture with pale yellow precipitate was observed. Afterwards, the mixture was left overnight at room temperature to guarantee the reaction stabilized and all nanoparticles precipitated. The resulting precipitate was separated using a centrifuge at 6000 rpm for half an hour. The sediment was splashed several times by a 2:3 solution of pure ethanol and deionized water to eliminate contaminants, and the mixture was centrifuged again to collect the washed precipitate. The precipitate was then dried in an oven at 60 °C for 24 hours. The sediment was then calcined at 450 °C for 2 hours, and the zinc oxide nanopowder was kept for later use in an opaque, airtight tube.

Characterization of Zinc Nanoparticles Synthesized from *B. vulgaris* L. Aqueous Extract

X-ray Diffraction (XRD)

In this test, X-ray diffraction was used to examine crystallite size, phase purity, and crystal structure in nanomaterials (Sharma *et al.*, 2026).

Field Emission Scanning Electron Microscope (FE-SEM)

This test offers more accurate plus clearer images for measuring the nanoscale dimensions and shapes of zinc oxide nanoparticles (Saware and Venkataraman, 2014). The analysis was conducted at Al-Khawra Company in the Baghdad Governorate using an inspect 50 (manufactured by FEI company, Netherlands).

Fourier-Infrared (FT-IR) Spectroscopy

To determine which active substances are in charge of the reduction and stabilization processes, infrared spectroscopy was performed on the plant-mediated ZnO-NPs within a wavelength range of 400-4000 cm^{-1} (Sharma *et al.*, 2026). The analysis was conducted at Al-Khawra Company in the Baghdad Governorate using a Fourier transform infrared spectrophotometer (manufactured by Perkin Elmer 1725x, Japan).

Antifungal Effect of Zinc Oxide Nanoparticles (ZnO NPs)

The antifungal effect of Zn-ONPs mediated by the extract of *B. vulgaris* L. was evaluated against three isolated fungi from three plants *in vitro*. The antifungal activity of ZnO-NPs was tested using the poisoned food technique (Kumawat *et al.*, 2025). Zinc oxide nanoparticles were prepared at concentrations of (25, 50, 100, and 200) ppm and subsequently incorporated into 200 mL of sterilized (PDA) medium. A control treatment was also used, including

plates containing only (PDA) medium without the addition of any other material.

The plates were vaccinated with a 5 mm diameter disc of the fungi under study taken from pure colonies aged 7 days, using three independent replicates for each treatment and the control group. The diameter of the developing colony was measured after seven days of incubation at 27 ± 2 °C (average of two perpendicular diameters) was measured. The percentage of growth inhibition was calculated using the equation (Vincent, 1947).

$$\text{Growth inhibition (\%)} = C - T / C \times 100$$

Where: C is the mean colony diameter in the control plates (mm), and T is the mean colony diameter in the treatment plates (mm).

Statistical Analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA) at a significance level of 0.05 and the Least Significant Difference (LSD) test for each fungus independently, with three replicates per concentration.

RESULTS AND DISCUSSION

Fungal Isolation

The results of the isolation and identification of fungi isolated from the roots of some plants infected with rot symptoms in various orchards in Karbala Governorate were presented. The isolated fungi were identified according to morphological characteristics such as colony color and tissue, and microscopic characteristics, especially the shape of the fungal hyphae, conidiophore, conidia, and conidia color, among others, when cultured on PDA medium for one week at 25-27 °C, according to approved taxonomic keys.

Alternaria Alternata

The diameter of the *Alternaria alternata* colony on PDA medium ranged from 50-70 mm, and sometimes this fungus completely covered the plate at a temperature of 25-27 °C, over the course of seven days. This colony is characterized by its dense, brown or gray color, and the back of the plate is dark brown or black; see (Figure1A). As for the microscopic morphology of the fungus, the mycelium appeared divided, and the conidia were arranged in long chains on short, irregularly branched stalks. The conidia may reach ten units. The conidia are characterized by being longitudinally and transversely divided, with a pear-shaped shape that is tapered at the apex, and their diameter is 20-40 micrometers, as shown in (Figure 1B). The outcomes agreed with the claims made by (Wouden, 2015).

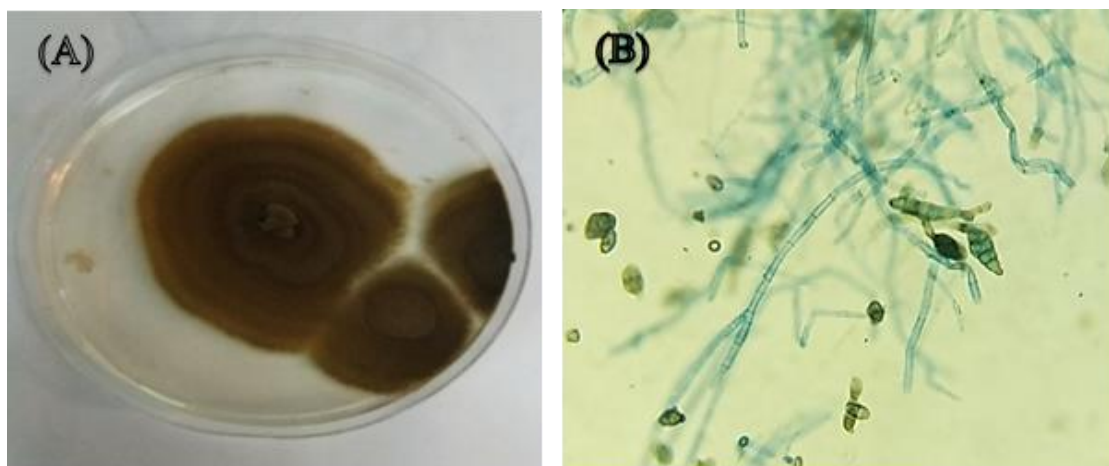


Figure (1): Morphological and Microscopic characteristics of *A. alternata*. (A) Colony of the fungus on PDA. (B) Mycelium and conidia

Rhizoctonia solani

The *Rhizoctonia solani* appeared in white colonies at the start of its growth and eventually turned dark brown instead of light brown. Constriction developed at the point of branching, and lateral branches developed at right angles to the mycelium's main branches. This quality is the most crucial morphological feature (Figure 2A). Microscopic characteristics were represented by the appearance of filaments divided by perforated septa. The sclerotia appeared as solid, cohesive, irregular, brown masses (Figure 2B). The outcomes agreed with the claims made by (Bradley and Ajayi-Oyetunde, 2018).

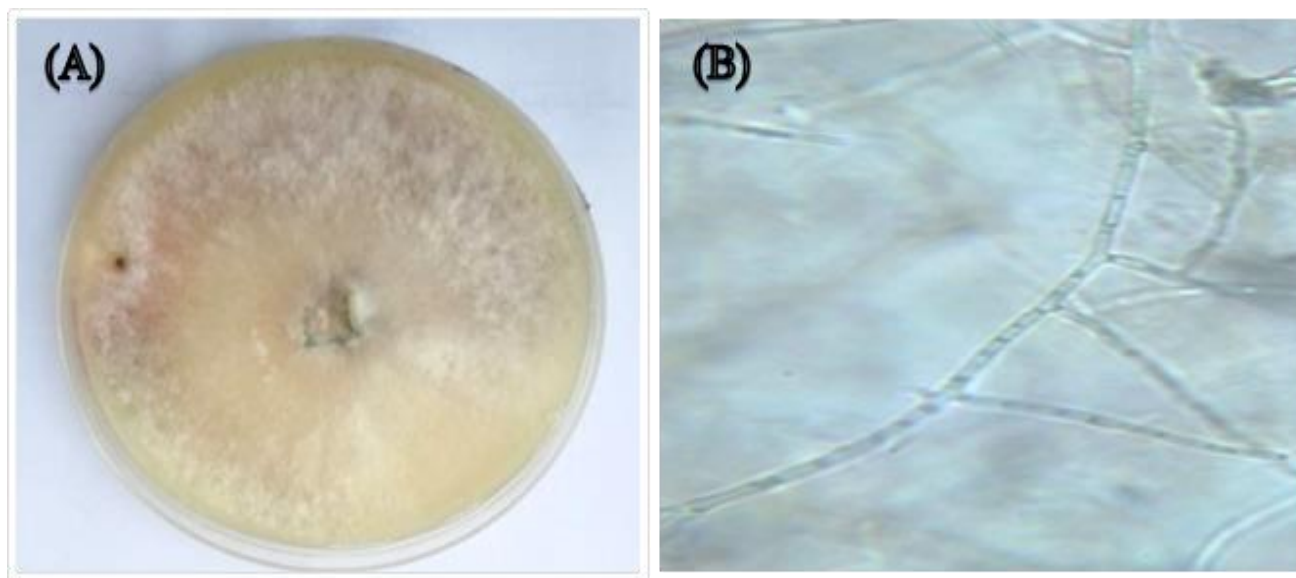


Figure (2) Morphological and Microscopic characteristics of *Rhizoctonia solani*. (A) Colony of the fungus on PDA. (B) Mycelium.

Fusarium Solani

The *Fusarium solani* produces white, aerial mycelium that turns light or dark pink depending on the pigments secreted in the culture medium in which the fungal colonies are growing. The color range varies between light and dark pink (Figure 3A). Microscopic characteristics include mycelium divided by simple septa and the presence of two types of conidia: macroconidia, crescent-shaped that are divided by 3-5 or more septa, and microconidia, oval or spherical, undivided or divided by only one or two septa (Figure 3B), as these results are consistent with (Muratali *et al.*, 2025).

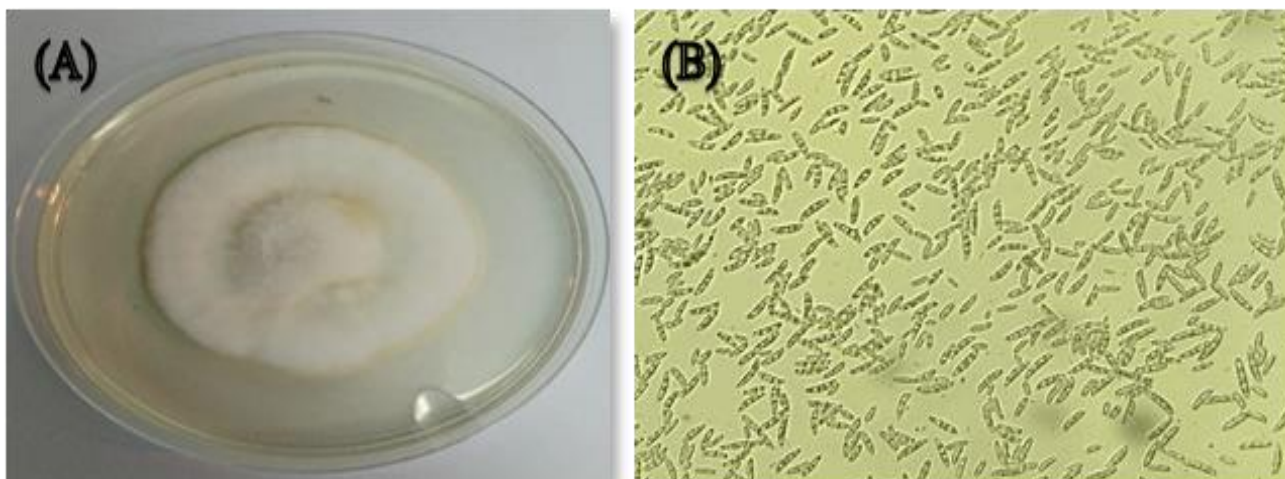


Figure (3) Morphological and Microscopic characteristics of *F. solani*. (A) Colony on PDA. (B) Macroconidia and Microconidia

Characterization of ZnO-NPs Biomediated

X-ray Diffraction (XRD)

The XRD pattern of ZnO-NPs displays a sequence of distinct, sharp diffraction peaks at various 2θ angles, indicating the material's crystalline quality (Figure 4). The main peaks appear at approximate 2θ values of (31.7°), (34.4°), (36.2°), (47.5°), (56.6°), (62.8°), (66.3°), (67.9°), and (69.1°), which can be attributed to crystal planes 100, 002, 101, 102, 110, 103, 200, 112, and 201 individually. The sharpness and high intensity of the peaks, especially the strongest peak at plane (101), indicate a high degree of crystallinity and regularity of the crystal lattice. X-ray diffraction results confirmed that the manufactured ZnO in a tested sample is consistent with crystalline hexagonal ZnO (Mobarak, 2025), making it suitable for biological, environmental, and physical applications that require stable structural properties and appropriate nanoparticle size (Klinbumrung *et al.*, 2022).

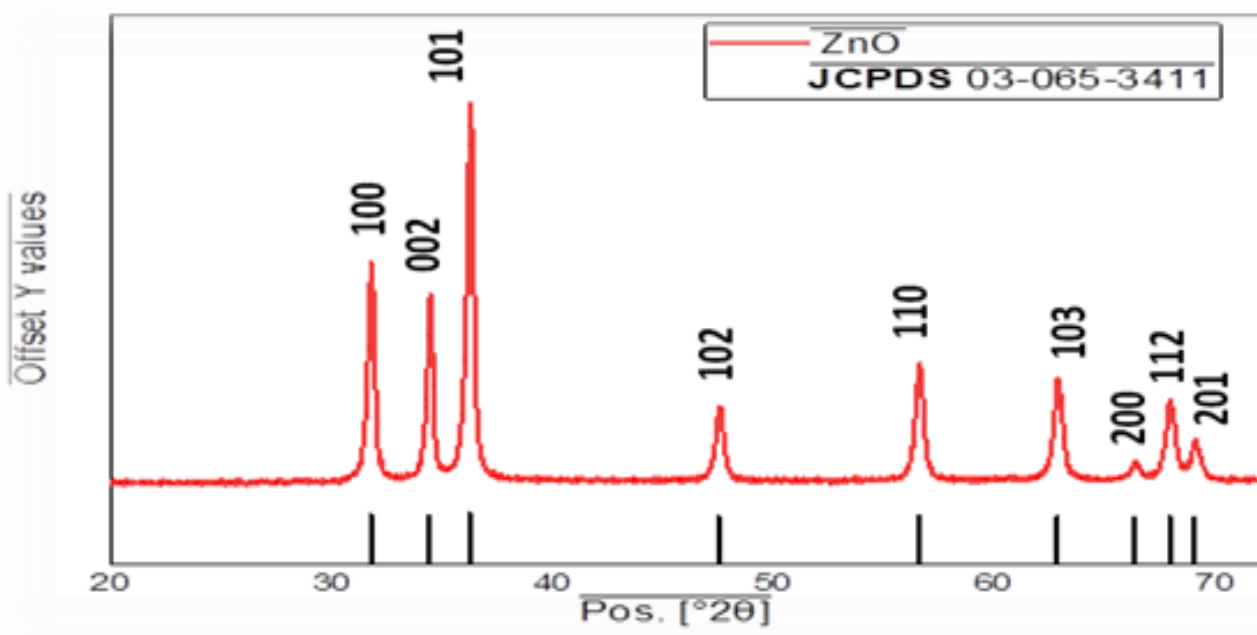


Figure (4) X-ray diffraction spectrum of ZnO-NPs synthesized from the aqueous extract of *B. vulgaris* L.

Field Emission Scanning Electron Microscopy (FESEM)

This test shows that the particles of ZNO possess an irregular nanomorphology tending towards a spherical shape, with a clear agglomeration phenomenon observed. This behavior is attributed to the high surface energy of the nanoparticles, as well as the role of bioactive compounds present in the extract of *B. vulgaris* L., such as flavonoids and phenols, which function as partial stabilizing and reducing agents during the preparation process. Direct measurements taken from FESEM images indicate that the particle diameters range approximately from 43 to 63 nm, with an average size of about 50 nm, as shown in Figure 5. This confirms that the particles are formed at the nanoscale with limited size variation. The relatively rough outer surface of the particles also suggests that the visible particles may consist of aggregates of several cohesive primary nanocrystals. This morphology may enhance their potential use in biological and environmental applications that require a high surface area and effective surface activity (Mousa *et al.*, 2024).

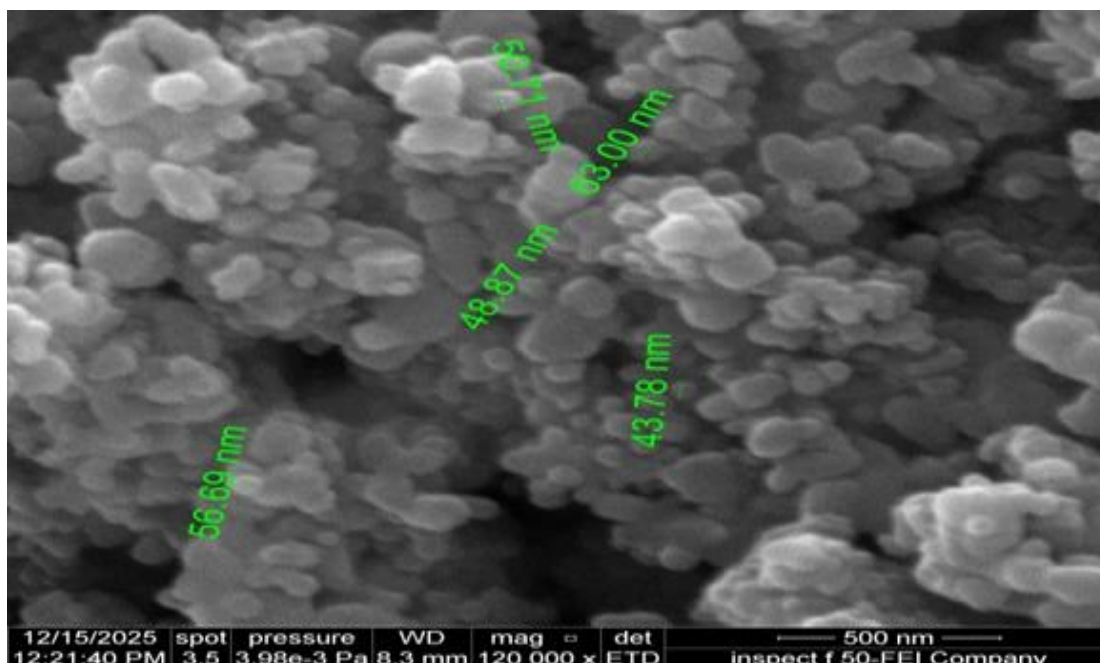


Figure (5) FE-SEM of ZnO-NPs synthesized by extract of *B. vulgaris* L.

FT-IR Spectroscopy

FT-IR spectroscopy was used to identify the functional groups linking the ZnO-NPs surface and to identify the characteristics of the chemical bonds formed during the preparation process. The results in Figure 6 showed broad absorption peaks at wavenumbers 3556, 3479, 3408, and 3232 cm^{-1} , indicating the presence of hydroxyl groups (O-H) or molecules of water on the exterior of the ZnO substance. These peaks can also reflect residues of plant compounds used in biopreparation that contribute to the stabilization of nanoparticles (Hameed *et al.*, 2023). (C-H) peaks also appeared at (2920-2850 cm^{-1}) which indicate residues of plant organic compounds that serve as reducing and encapsulating agents (Umar, 2026), supporting the stability of nanoparticles and preventing their aggregation. As for the characteristic peaks in the low-frequency region (430-550 cm^{-1}) which are due to the stretching vibration of the (Zn-O) link (Mostafa, 2025), mirrors clear evidence of successful formation of zinc oxide nanoparticles.

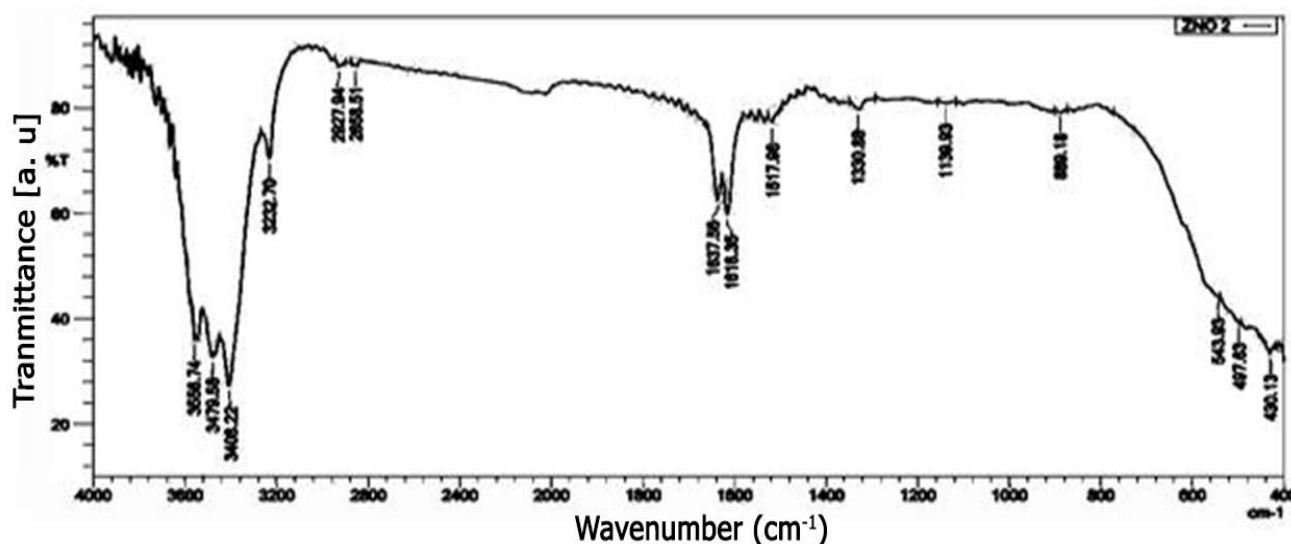


Figure (6) FT-IR Spectrum of ZnO-NPs synthesized by the aqueous extract of *B. vulgaris* L.

Evaluation of the Antifungal Activity of Zinc Oxide Nanoparticles Synthesized using Aqueous Extract of the *Beta vulgaris* L.

The study's findings revealed a direct relationship between the concentration of the aqueous nano-extract of the *B. vulgaris* L. and its inhibitory activity against the fungi isolated from the roots of infected plants. The diameters of colonies were gradually decreased with increasing concentration ZnO-NPs. The concentration of 200 ppm recorded the highest Inhibitory effect compared to the other concentrations used (25, 50, and 100 ppm), with a clear difference in the degree of response between the fungal species, and with a statistical analysis confirming significant differences between concentrations for each species ($P \leq 0.05$). This indicates the increased efficiency of ZnO-PNs at higher concentrations in reducing the growth of the tested fungi, as shown in the Figure 7.

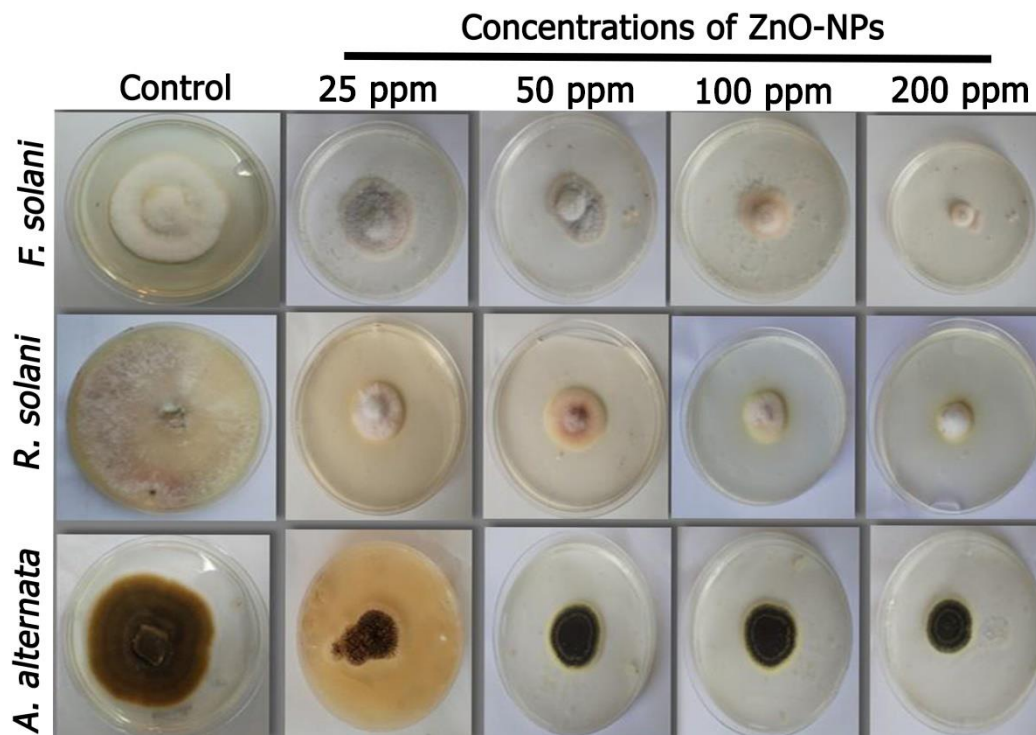


Figure (7) Zinc oxide nanoparticles' impact on the fungal isolates associated with plant roots rot.

These results are consistent with the studies of (Yassin *et al.*, 2025, Krumova *et al.*, 2024), which demonstrated the efficacy of synthesized zinc oxide nanoparticles in inhibiting the growth of various fungal species, even at low dosages. This action is explained by ZnO nanoparticles' capacity to increase reactive oxygen species generation, which oxidizes membrane proteins and lipids. This causes cellular components to leak out, and it upsets fungal cells' homeostasis (Xu *et al.*, 2023). The results presented in table (1) revealed differences in the fungal response to the extract. *A. alternata* was the most sensitive fungus at a concentration of 200ppm, recording the highest inhibitory percentage (85.56%) compared to the other fungi. In contrast, *R. solani* recorded the lowest inhibitory percentage at 80.00 %. These differences may be attributed to variation in fungal species and the physiological characteristics of the cell walls. The higher sensitivity of some fungal species may be related to their limited ability to tolerate nanoparticles (Abdulqudos and Abdulrahman, 2022). These nanoparticles may cause disturbance in the fungal cell membrane and cell wall and interfere with vital processes required for fungal growth. Furthermore, the nano-formulation enhanced the inhibitory efficiency due to its permeability and ability to reach fungal cells.

Table (1) Effect of the inhibitory activity of zinc oxide nanoparticles (ZnO) from *Beta vulgaris* aqueous extract on the Mean of colony diameter (mm) of fungal isolates associated with plant roots.

Types Fungi	Control	Inhibitory percentage (%)				LSD
		25 ppm	50 ppm	100 ppm	200 ppm	
<i>Alternaria alternata</i>	90	66.67	68.89	75.56	85.56	0.94
<i>Rhizoctonia solani</i>	85	65.88	68.24	76.47	80.00	1.05
<i>Fusarium solani</i>	90	61.11	64.44	72.22	83.33	4.77

CONCLUSION

The aqueous extract of *B. vulgaris* L. prepared via green synthesis demonstrated efficacy in producing zinc oxide nanoparticles. Fungi causing root rot, specifically *Alternaria alternata*, *Rhizoctonia solani*, and *Fusarium solani* were isolated and identified. The zinc oxide nanoparticles exhibited inhibitory activity against the isolated fungi. The highest inhibitory effect was recorded at a concentration of 200 ppm, with the response varying according to the fungal species.

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