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RESEARCH ARTICLE

Seed Treatment-Based Biocontrol of Tomato Damping off Using Plant Essential Oil and Green Silver Nanoparticles

Laila Reklaoui[1], Zine El Abidine Bzazou Elouazzani[1], El Touhami Tayebi Ouazzani[1], Mariam El Idrissi El Berkani[1], Ferdaous Dmini[1], Abderazzak Rfaki[2], Hassan Ghazal[2], Haiat Essalmani[1], Said Barrijal[1]

[1]Laboratory of Biotechnological Valorization of Microorganisms, Genomics, and Bioinformatics, Faculty of Sciences and Techniques, Abdelmalek Essaadi University, Tangier, Morocco
[2]National Center for Scientific and Technical Research CNRST, Rabat 10000, Morocco

*Corresponding Author: l.reklaoui@uae.ac.ma

Abstract: The fungal pathogen *Rhizoctonia solani* is a major causal agent of damping-off in tomato plants, leading to severe yield losses worldwide. Conventional fungicides, while effective, raise concerns regarding environmental safety and human health, highlighting the urgent need for sustainable alternatives. In this study, we report for the first time the use of *Origanum compactum* essential oil to biosynthesize silver nanoparticles and evaluate their antifungal efficacy against *Rhizoctonia solani* in tomato. This novel approach integrates Moroccan endemic plant resources with nanotechnology for sustainable disease control under both laboratory and greenhouse conditions. The synthesized AgNPs were characterized using UV–Vis spectroscopy, SEM, XRD, and EDX analyses. Tomato seeds were treated with AgNPs and EO and evaluated for germination rate, disease incidence, and seedling growth parameters. Seed germination assays showed that short immersion (2 h) in AgNPs at 50 ppm maintained high germination (92% vs. 85% in control, $p < 0.05$), whereas prolonged exposure (24 h) completely inhibited germination (0%), confirming dose- and time-dependent phytotoxicity. In *vitro* assays revealed that AgNPs at 50 ppm inhibited *R. solani* growth by 93.7%, while EO achieved 62.5% inhibition. Under greenhouse conditions, AgNPs at 50 ppm provided the highest disease suppression (86.5%) and significantly enhanced root/shoot growth and overall seedling vigor. These findings suggest that green-synthesized AgNPs, particularly in combination with EO, represent a promising eco-friendly alternative to conventional fungicides. Further validation under field conditions, along with studies on the molecular mechanisms involved, will be essential to optimize their application in integrated disease management strategies.

Keywords: Damping Off, Silver Nanoparticles, *Origanum compactum*, Essential Oil, *Rhizoctonia solani,* Antifungal Activity, Biological Control

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Introduction

Tomato (*Solanum lycopersicum L.*) ranks as the second most consumed vegetable worldwide, surpassed only by potatoes [1]. Due to its adaptability to diverse climates and its high economic importance, tomato plays a crucial role in global

agricultural production systems and food security. Botanically classified as a fruit but regarded as a vegetable in culinary contexts, tomatoes are cultivated both in greenhouses and in open fields. Consumption includes fresh tomatoes as well as processed forms like pastes, purées, canned goods, juices, and dried varieties. Between 2018 and 2022, an average of 4,972,265.6 hectares of tomato fields produced approximately 184,940,640.7 tons annually worldwide [2]. In Europe alone, 415,163.4 hectares yielded an average of 22,833,989.89 tons per year. Major European producers include Turkey, Italy, and Spain all within the top ten global tomato-producing countries. In Morocco, tomato production averaged 1.39 million tons annually during the same period [2].

Despite advancements in agricultural practices, tomato crops remain vulnerable to numerous pathogens that cause diseases such as root rot, stem canker, and wilt, significantly reducing yield and quality. Among these pathogens, *Rhizoctonia solani* is notably aggressive. This soil- borne fungus persists for extended durations in plant debris and flourishes under both tropical and temperate climates. Unlike many fungi, R. solani does not produce asexual spores (conidia) but instead forms survival structures called sclerotia, which allow it to persist in adverse conditions [3]. R. solani is responsible for damping-off disease, characterized by seed rot or seedling death prior to emergence (pre-emergence damping-off) and post- emergence seedling collapse (post-emergence damping-off), leading to yield losses of up to 50% [4]. This disease affects tomato production in both field and greenhouse environments.

Currently, there are no commercially available tomato cultivars resistant to R. solani, making chemical fungicides the primary method of control. However, reliance on synthetic fungicides presents significant challenges. The repeated application of synthetic fungicides often leads to contamination of soil and water, and their use can release particles into the air during spraying. This not only affects ecosystems but also encourages the emergence of resistant fungal populations. According to [5], as much as 60–70% of fungicides may be lost through drift or surface runoff during treatment. In addition, extended reliance on these chemicals raises serious concerns for environmental sustainability and public health [6].

Nanotechnology offers transformative prospects for agriculture through the development of advanced solutions in disease control, early pathogen detection, and improved nutrient delivery to plants. It introduces safer and more efficient techniques for applying agrochemicals, with minimal environmental impact. For example, nanoscale carrier systems allow precise and controlled release of pesticides at specific target sites, significantly lowering the risk of environmental pollution [7].

Growing concern over the environmental and health hazards linked to synthetic fungicides has prompted researchers to explore more sustainable options. Among the earliest nanomaterials investigated, silver nanoparticles (AgNPs) have shown strong antimicrobial effects and have been effective against a broad spectrum of plant pathogens, including fungal species [8]. Thanks to their wide-ranging properties mechanical strength, optical behavior, antimicrobial action [9], as well as electrical and thermal conductivity [10, 11]. AgNPs are considered promising candidates for managing plant diseases.

As global food production needs are expected to rise sharply in the coming decades, AgNPs could play a significant role in improving soil conditions [12], increasing pesticide efficacy, and promoting plant health and growth [13]. Their impact, however, can vary depending on several factors, including particle size, type, and dosage [14]. Other elements like plant variety, cultivation environment, soil characteristics, and nanoparticle bioavailability also influence their overall effectiveness. For this reason, it is critical to assess how AgNPs affect food crops before considering their widespread agricultural use [15].

The synthesis methods for nanomaterials are critical for determining their biological applications. Approaches can be classified into physical, chemical, and biological categories. Physical methods include techniques such as physical vapor deposition, ball milling, sol-gel processes [16], laser ablation, and electron beam evaporation [17]. These methods often require expensive equipment and infrastructure, have limited reproducibility due to sensitivity to process parameters, and generate hazardous waste that requires proper disposal [18].

Chemical synthesis techniques, such as hydrothermal synthesis, coprecipitation, chemical reduction, microemulsion, and solvothermal synthesis, often involve costly synthetic chemicals and produce hazardous by-products [19].

Biological methods, which utilize microorganisms such as bacterial and fungal cell cultures [20], or plant-based systems [21], have emerged as a sustainable alternative. Microorganisms promote plant growth and nutrient availability through several mechanisms, including the exudation of organic acids, chelation of mineral ions, production of siderophores, and effective colonization of the rhizosphere, which enhance nutrient solubilization and plant health. Compared to other biological sources, plants are preferred due to their simple, one-step procedure that eliminates the need for complex cell culture maintenance and aseptic environments [22]. Additionally, plants are widely available, easily sourced locally, and contain diverse metabolites that act as reducing and capping agents for nanoparticle formation, thereby preventing undesirable by-

products and enhancing nanoparticle stability [23]. Green synthesis offers a sustainable route for producing nanoparticles with desirable features, including improved stability, strong antibacterial activity, and high biocompatibility, while also being cost-efficient and environmentally friendly [24]. This process involves the reduction of silver ions (Ag^{+}) to metallic silver nanoparticles (Ag^{0}) by plant-derived metabolites, such as phenolics and terpenes, which also act as stabilizing agents. Using plants for nanoparticle fabrication has gained attention for its versatility, with applications spanning agriculture, medicine, environmental cleanup, and energy. Unlike traditional chemical synthesis, which often relies on hazardous substances, plant-based methods offer a safer and more natural alternative [25]. In particular, essential oils have been recognized as effective agents in this process, thanks to their rich content of bioactive molecules such as terpenes and phenolic compounds, which exhibit strong antimicrobial potential [26]. Therefore, green synthesis not only reduces environmental risks but also provides a promising perspective for the development of eco-safe biopesticides and sustainable nanotechnology applications.

Among the essential oils, *Origanum compactum Benth,* commonly known as Moroccan oregano, was used for essential oil extraction. *Origanum compactum* EO stands out for its potent antimicrobial activity. Its bioactive constituents, including carvacrol and thymol, exhibit significant antifungal effects, making it an ideal candidate for nanoparticle synthesis. Previous studies have demonstrated that metal nanoparticles synthesized using plant extracts; including silver nanoparticles, have applications beyond agriculture, extending to fields such as electrochemistry, catalysis, optics, and biomedicine. In agriculture, these nanoparticles offer promising avenues for sustainable plant protection [8].

Given these considerations, this study aimed to:

(i) Develop a green synthesis method for silver nanoparticles (AgNPs) using *O. compactum* essential oil (EO)

(ii) Evaluate the efficacy of these AgNPs as a nano-fungicide against *R. solani*, the causal agent of damping-off disease in tomato plants

(iii) Assess the impact of AgNPs and EO on seed germination, disease incidence, and seedling vigor

(iv) Determine the optimal seed immersion time for effective disease control without causing phytotoxicity

(v) Compare the effectiveness of AgNPs and EO treatments with pathogen-infected and untreated control groups. By integrating nanotechnology and plant- based approaches, this research seeks to provide a sustainable and efficient alternative to chemical fungicides, contributing to the development of eco-friendly disease management strategies for tomato crops. The findings are expected to pave the way for broader applications of AgNPs in sustainable agriculture

Materials and Methods

Fungal Source and Growth Conditions

Rhizoctonia solani collected from stock cultures (mycotheque) of the Laboratory of Biotechnological Valorization of Microorganisms, Genomics, and Bioinformatics, Department of Biology, Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaadi University, Morocco. This isolate has been previously characterized in our laboratory based on its characteristic colony morphology, hyphal features observed microscopically, and pathogenicity tests following Koch's postulates as reported [27]. The fungal isolate was maintained on Potato Dextrose Agar (PDA) medium at 25 ± 2°C for 7 days to promote mycelial growth. Stock cultures were stored at 4°C and periodically sub-cultured to ensure viability.

Pathogenicity Test

In vitro Pathogenicity Test

The pathogenicity of R. solani was assessed using radicle assay in petri dishes, according to [28], with some modifications. Tomato seeds (the Campbell 33 variety was chosen for the experiment) were surface sterilized with 4% sodium hypochlorite for 15 minutes and rinsed three times with sterile distilled water. 0.2% water agar plates were prepared and inoculated at the center with a 5 mm diameter disc of actively growing R. solani mycelium. Four seeds were aseptically and equidistantly placed around the fungal disc and incubated at 25°C for 5 days. Plates contained sterile PDA disc serve as control. The disease progression was assessed based on the extent of the radicle necrosis area and the average radicle length, following the scale described by recent studies [29, 30].

Pathogenicity Test Under Greenhouse Conditions

A greenhouse pathogenicity test was carried out in small plastic pots containing sterilized soil. To produce the fungal inoculum, barley-based medium (100 g of barley supplemented with 1 g of yeast extract, 2 g of glucose, and 100 mL of distilled water) was autoclaved and inoculated with five 5 mm discs from a 5-day-old culture of *Rhizoctonia solani*. The inoculated flasks were gently shaken by hand each day to prevent clumping. After 15 days of incubation at 24°C, the medium was fully colonized by the fungus. The pots were filled with a sterilized soil mixture composed of field soil, sand, and peat in a 3:1:1 ratio [30]. This mixture had been sterilized beforehand by autoclaving at 121°C for one hour on two consecutive days. The colonized barley grains were then evenly mixed into the sterile soil at a concentration of 1% (w/w). Seven days after introducing the pathogen to the soil, surface-sterilized tomato seeds were planted. The pots were kept under controlled greenhouse conditions (25 ± 2°C with a 12- hour light/dark cycle). Control pots received sterile barley instead of the fungal inoculum. Pots were arranged in a completely randomized design and rotated regularly to minimize positional effects. Each treatment was performed in triplicate, and the experiment was independently repeated twice to ensure reproducibility. After another seven days, germination was assessed using the following formula:

Germination Rate (%) = (Number of germinated seeds / Total seeds planted) ×100

Biosynthesis of AgNPs Using Origanum Compactum Essential Oil

Preparation of Essential Oil

The essential oil of *Origanum compactum Benth*, was extracted using hydrodistillation for 3 hours with a Clevenger apparatus, a standard device that condenses and separates volatile oils from plant material. Fresh plant material (200 g) was distilled with 1 L of distilled water. The oil was separated, and stored at 4°C in a dark glass vial until use.

Formation of AgNPs

The procedure outlined was adopted with slight adjustments [26]. Initially, the essential oil was diluted in acetone at a ratio of 1:170 (v/v) to ensure rapid integration into the aqueous medium. For nanoparticle synthesis, a 1 mmol/L silver nitrate ($AgNO_3$) solution was prepared and adjusted to pH 8, since an alkaline environment favors the reduction of Ag^+ ions. Subsequently, 2 mL of the diluted essential oil was added drop by drop into 20 mL of the $AgNO_3$ solution under vigorous stirring at 70 °C for one hour. The phytochemicals present in the essential oil, mainly phenolic compounds such as pulegone, borneol and carvacrol, acted as reducing agents, converting Ag^+ ions into metallic silver (Ag^0), while simultaneously serving as capping agents to stabilize the nanoparticles and prevent aggregation. A visible color change from clear to brown confirmed the successful reduction and formation of AgNPs. Once the reaction was complete, the nanoparticles were collected by centrifugation at 10,000 rpm for 10 minutes, and the resulting pellet was stored for further characterization.

AgNPs and O. Compactum Essential Oil Efficacy on the Growth of the Fungi in vitro

The antifungal activity of silver nanoparticles (AgNPs) and Origanum compactum Essential Oil (EO) was evaluated in vitro against *Rhizoctonia solani* using the poisoned food technique [27] (Reklaoui et al., 2024). Petri dishes (9 cm in diameter) containing (PDA) potato dextrose agar were supplemented with five concentrations of AgNPs and EO (12.5, 25, 50, 100, and 150 ppm) to determine the Minimum Inhibitory Concentration (MIC). A 5 mm agar plug from a pure culture of R. solani was placed at the center of each plate using a sterile cork borer. Control plates contained PDA without AgNPs or EO. All plates were incubated at 25°C for 7 days, and the experiment was performed in triplicate. To prevent contamination, all procedures were conducted in a laminar airflow cabinet under sterile conditions.

The radial growth of the colony was measured, and the rate of inhibition was calculated using the following formula:

$$\text{Rate of inhibition } (\%) = \frac{R - \mathrm{r}}{R} \times 100 \quad (1)$$

Where *R* = Radial growth of *R. solani* in the control plates (mm) and r = Radial growth of *R. solani* in AgNP- or EO-treated plates (mm).

Characterization of the Biosynthesis AgNPs

The suspension samples were analyzed using a SHIMADZU UV-Vis spectrophotometer across a wavelength range of 200 to 1100 nm to capture absorbance profiles. Structural characteristics of the nanoparticles were examined via X-ray

diffraction (XRD) using a Bruker D8 ADVANCE diffractometer with CuKα radiation (λ = 1.5406 Å), scanning across a 2θ range from 10° to 80°. Raman spectroscopy was conducted with a Bruker SENTERRA II confocal Raman microscope, employing a 532 nm excitation wavelength and a 10-mW laser power. A Thermo Scientific Quattro Scanning Electron Microscope (SEM) was employed to assess particle size. Compositional analysis was further carried out using energy dispersive X-ray spectroscopy (EDS).

In vitro INHIBITIOn of Tomato Damping-off by AgNPs and EO at 50 ppm

The minimum inhibitory concentration (50 ppm) of AgNPs was determined by testing their ability to prevent seed infection *in vitro* [31]. Since the MIC of EO is higher than 50 ppm, EO was tested at 50 ppm for comparison purposes. Seeds were sterilized for 15 min using 4 % sodium hypochlorite, and then they were washed three times with sterile water. Tomato seeds were immersed in AgNPs (50 ppm) and EO (50 ppm) for 2 hours. Seeds soaked in distilled water serve as control. Treated and untreated seeds were sown on water agar 0.2% plates inoculated with *R. solani*. Germination rates and radical length were recorded after 5 days.

Inhibition of Tomato Damping-off by AgNPs and EO at 50 ppm Under Greenhouse Conditions

The inhibitory effect of AgNPs and Essential oil of *Origanum compactum* on damping off diseases in tomato was evaluated at 50 ppm, with modifications [32]. Pots containing sterilized soil were inoculated with barley grains colonized by *R. solani* at a rate of 1% (w/w) as mentioned previously. Seeds of the Campbell 33 variety, previously sterilized with sodium hypochlorite, were planted at a rate of ten seeds per pot after being immersed for 24 hours, and 2 hours in either AgNPs, EO or distilled water as a control. Seeds grown in a soil pot containing sterile soil without pathogens were used as a negative control. The disease incidence of pre-and post-emergence of tomato plants were recorded after 15 and 30 days respectively. The experiment was monitored daily, and observations were recorded 30 days after planting, including Germination Rates (Gr), Disease Incidence (DI), Control Efficacy (CE), Root Length (RI), Shoot Length (SI), Fresh weight (Fw), Dry weight (Dw), and VIGOR Index (VI) [30]. A total of 30 seeds (three pots) were used for each treatment, and the experiment was repeated twice.

DI (%) = (Number of infected seedling/ Total seedlings) ×100

CE (%) = (DI of Pathogen Control −DI of Treatment)/ DI of Pathogen Control ×100

VI = Germination (%) × Mean Seedling Length (cm), where mean seedling length = root + shoot length.

All treatments were performed in triplicate, and each experiment was independently repeated twice to ensure reproducibility. Data are expressed as mean ± Standard Deviation (SD).

Statistical Analysis

All treatments were performed in triplicate, and each experiment was independently repeated twice. Data were analyzed using one-way analysis of variance (ANOVA), and differences among means were determined using Tukey's post hoc test at a 5% significance level. Results are expressed as mean ± Standard Error (SE). All statistical analyses were performed using Excel STAT software (version 2014).

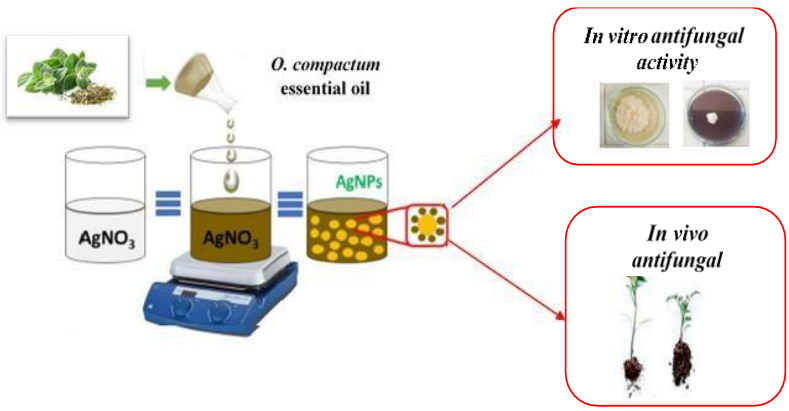

Fig. 1: Graphical illustration of the synthesis of silver nanoparticles using essential oil of *Origanum compctum*

The overall experimental procedure used in this study illustrated in Figure 1. The essential oil of *O. compactum* was employed as both a reducing and stabilizing agent for the synthesis of silver nanoparticles from $AgNO_3$ solution. The biosynthesized AgNPs were subsequently evaluated for their antifungal activity through *in vitro* assays against *Rhizoctonia solani* and in vivo tests on tomato seedlings. This schematic representation summarizes the workflow of the study, from nanoparticle synthesis to antifungal assessment.

Results

Pathogenicity Test

The pathogenicity of *Rhizoctonia solani* was confirmed both *in vitro* and under greenhouse conditions. The pathogenicity assay confirmed that *R. solani* was highly virulent on tomato seeds, causing extensive radicle necrosis under *in vitro* conditions, whereas no symptoms were observed in the control treatment (Figure 2). The radicle necrosis rate reached approximately 75%, highlighting the strong pathogenic potential of *R. solani* on tomato seeds. The average radicle length of tomato seeds inoculated with *R. solani* was 23.5 mm, which represents a significant reduction compared to 50.5 mm in the non-inoculated control ($p < 0.05$). These findings are consistent with previous studies where the pathogenicity of *R. solani* was assessed on chickpeas and beans using a radicle assay, similarly showing severe necrosis and significant reductions in radicle length under *in vitro* conditions [28].

Fig. 2: Tomato seeds: control (left); infected with *R. solani* (right)

The pathogenicity test conducted under greenhouse conditions demonstrated that *R. solani* significantly reduced the germination rate of tomato seeds as shown in Figure 3. In inoculated pots, the germination rate was only 13%, compared to 70% in non-inoculated control pots, highlighting the strong pathogenic potential of *R. solani*. Many seeds in the inoculated soil failed to germinate, exhibiting signs of decay such as darkened seed coats and softened tissues, which are characteristic of fungal infection. In contrast, seeds in the control pots germinated normally, with no visible symptoms of infection.

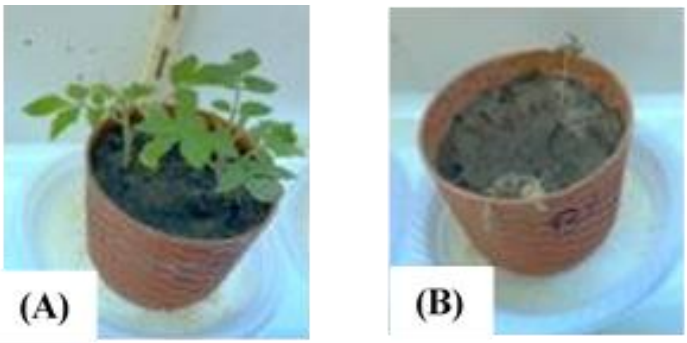

Fig. 3: Pathogenicity assay of *R. solani*, using the soil infection method under greenhouse conditions. (A) Pot untreated by *R. solani* (B) Pot treated by *R. solani*

The characteristic symptoms of root rot caused by *R. solani* in tomato seedlings under greenhouse conditions are clearly illustrated in Figure 4. The visible lesions observed on the stem base highlight the extent of fungal invasion, causing significant structural damage. Additionally, the evident brown discoloration of the primary root indicates tissue necrosis, impairing nutrient and water uptake, ultimately compromising the overall health and survival of the affected seedlings.

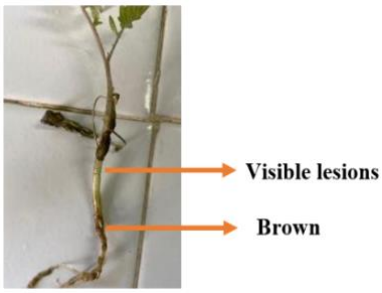

Fig. 4: Typical symptoms of tomato seedling root rot induced by *R. solani* under greenhouse conditions

Biosynthesis and Characterization of AgNPs With O. Compactum

The successful green synthesis AgNPs using *Origanum compactum* EO was confirmed by a color change from transparent to brown, indicative of silver ion reduction.

The phytosynthesis of silver nanoparticles was preliminarily assessed using UV-Vis spectrophotometry, which is a key technique for confirming nanoparticle formation. The resulting spectrum (Figure 5d) exhibited a strong and distinct absorption peak at 428 nm, confirming the presence of AgNPs due to Surface Plasmon Resonance (SPR). The peak position aligns with previous reports indicating AgNPs typically exhibit SPR bands between 400 and 450 nm, with variations depending on factors such as particle size, shape, and aggregation state. This was indeed confirmed by other studies [33, 34]. The observed SPR band further indicates the formation of colloidal AgNPs with a relatively uniform size distribution. The shift and intensity of the SPR band are influenced by the dielectric environment, capping molecules, and possible nanoparticle interactions, which may contribute to the stabilization of the prepared AgNPs [35].

Silver nanoparticles synthesized using Origanum compactum essential oil were characterized using Scanning Electron Microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The analysis revealed that the AgNPs were predominantly spherical and well dispersed, with sizes ranging from 5 to 25 nm, and the majority falling between 14 and 20 nm (Figure 5a, b). The SEM images showed a uniform distribution of particles, which is likely due to the presence of bioactive compounds in the essential oil acting as natural capping agents. These compounds may stabilize the nanoparticles through mechanisms such as hydrogen bonding and electrostatic interactions between their functional groups and the nanoparticle surfaces, promoting both formation and stability [36]. Similar nanoparticle shapes and distributions have been documented in previous studies involving green synthesis using plant extracts [33, 37].

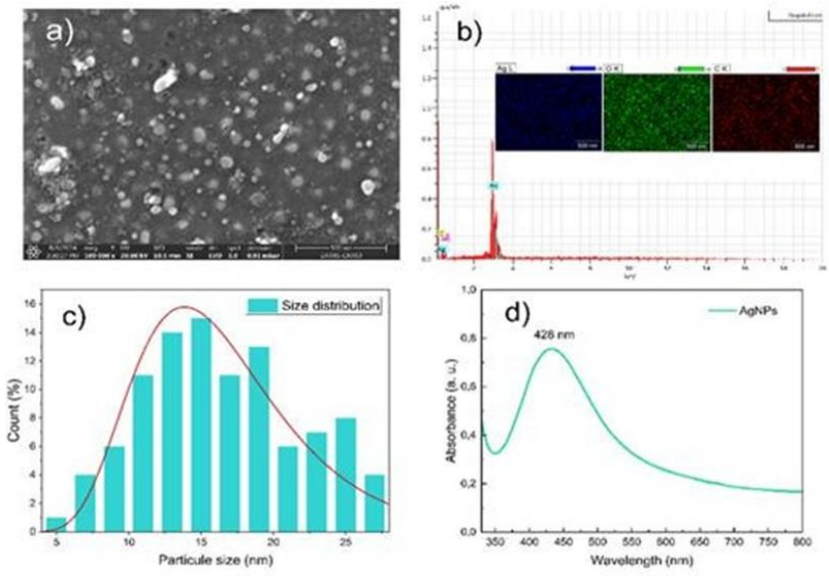

Fig. 5: (a) SEM images of biosynthesized AgNPs, (b) EDX spectrum with elemental mapping (Ag, O, C), (c) Particle size distribution histograms with lognormal fits (brown solid lines), and (d) UV-Vis absorption spectrum of AgNPs synthesized using *Origanum compactum* essential oil

In addition, EDX analysis (Figure 5c) verified the elemental makeup of the synthesized nanoparticles, showing prominent silver (Ag) signals near 3 keV. These findings confirm the presence of silver and are consistent with elemental patterns observed in prior studies [28, 38]. Additionally, signals corresponding to oxygen (O) and carbon (C) were detected in the range of 0.0–2.6 keV, indicating the presence of residual organic compounds from *O. compactum* EO on the nanoparticle surfaces. This suggests a dual role of Phyto molecules as both reducing and capping agents. The elemental mapping further demonstrated a uniform distribution of Ag, O, and C, supporting the involvement of bioactive molecules in nanoparticle stabilization.

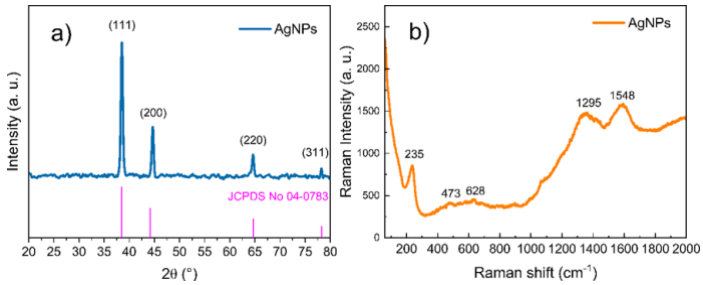

Fig. 6: (a) X-ray diffraction (XRD) patterns of biosynthesized silver nanoparticles, (b) Raman scattering spectra of synthesized silver nanoparticles

The XRD analysis of the synthesized AgNPs (Figure 6a) confirms their crystalline nature, as evidenced by the distinct diffraction peaks observed. The major reflections at 38.11° (111), 44.27° (200), 64.42° (220), and 77.47° (311) correspond to the characteristic peaks of face- centered cubic (fcc) silver nanoparticles, in agreement with the standard JCPDS No. 04-0783. The most intense peak at 38.11° suggests preferential growth along the (111) plane, indicating a dominant crystallographic orientation. A slight shift in peak positions compared to standard silver is observed, which may be attributed to the interaction of residual phytochemicals from *O. compactum* essential oil with the nanoparticle surface. Such shifts have also been reported in biosynthesized silver nanoparticles from other plant extracts. The crystallite size, calculated using the Debye-Scherrer equation, was estimated to be approximately 14.89 nm, indicating the formation of nanometer-scale silver particles. These results further support the successful synthesis of AgNPs with a well-defined crystalline structure [39, 40].

Surface-enhanced Raman spectroscopy (SERS) is a well-established technique for characterizing metallic nanoparticles, particularly silver nanoparticles. The enhancement of Raman scattering by colloidal AgNPs in liquid environments was first reported by [41], marking a significant milestone in plasmonic research. Since then, extensive studies have been conducted to optimize the size, shape, and surface properties of AgNPs, as these factors play a crucial role in their optical, chemical, and physical behavior.

Figure 6b presents the Raman spectrum of AgNPs synthesized using *O. compactum* EO. The spectrum reveals characteristic vibrational modes at 235, 473, 628, 1295, and 1548 cm^{-1}. A distinct peak at 235 cm^{-1} corresponds to Ag–O stretching vibrations, suggesting bonding interactions between silver and oxygen-rich functional groups derived from the essential oil. The absorption bands located at 473 and 628 cm^{-1} are associated with the stretching of C–N–C and C–S–C bonds, respectively, indicating the adsorption of nitrogen- and sulfur-containing organic molecules on the surface of the AgNPs [42]. Additionally, strong signals observed at 1295 and 1548 cm^{-1} can be linked to the symmetric and asymmetric stretching modes of carbonyl (C = O) groups, which are characteristic of carboxylic acid functionalities [43]. These spectral features collectively confirm the active role of essential oil-derived compounds in both stabilizing and functionalizing the silver nanoparticles.

Efficacy of AgNPs and EO on R. Solani in vitro

In this study, AgNPs were synthesized using a green approach, with *O. compactum* EO serving as both a reducing and stabilizing agent. The antifungal activity of these biosynthesized AgNPs was evaluated against *Rhizoctonia solani*, a major phytopathogen responsible for significant agricultural losses, particularly in tomato cultivation. Antifungal efficacy was assessed using the poisoned food technique, revealing a dose-dependent inhibition of *R. solani* by both EO and AgNPs. Notably, AgNPs exhibited significantly higher inhibition rates than EO alone, demonstrating a potential synergistic interaction between the two agents.

At 50 ppm, AgNPs achieved 93.7% inhibition, whereas EO alone required ≥100 ppm to reach a comparable level of effectiveness as shown in Table 1 and Figure 7. This substantial improvement highlights the superior antifungal potential of AgNPs, suggesting their promising application as potent biofungicides for controlling *R. solani.*

Table 1: Percentage of radial growth inhibition of *Rhizoctonia solani* by Essential Oil (EO) and silver nanoparticles (AgNPs) at different concentrations in (ppm). Values are expressed as mean ± SD (n = 3). Different letters (a–d) within the same column indicate significant differences according to Tukey's test at p ≤ 0.05

Percentage of radial growth inhibition of R. solani in %		
Concentration in ppm	**EO**	**AgNPs**
0	0.00 ± 0.00	0.00 ± 0.00
12.5	21.80 ± 1.33a	48.32 ± 1.26c
25	33.33 ± 1.26c	68.91 ± 0.73bc
50	62.50 ± 0.73bc	93.70 ± 0.00d
100	81.70 ± 0.17cd	93.70 ± 0.00d
150	85.70 ± 0.00d	93.70 ± 0.00d

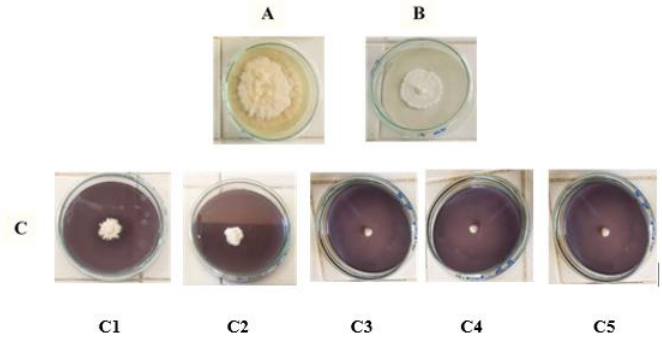

Fig. 7: Inhibition effect of *R. solani* mycelial growth: (A) Control without EO and AgNPs, (B) *O. compactum* EO at concentration 50 ppm, (C1–C5) AgNPs at 12.5, 25, 50, 100, and 150 ppm, respectively

In vitro Inhibition of Tomato Damping-off by AgNPs and EO at 50 ppm

The Minimum Inhibitory Concentration (MIC) of AgNPs was determined to be 50 ppm. In contrast, the MIC of the Essential Oil (EO) was higher than 50 ppm, indicating that higher concentrations are required to completely inhibit fungal growth. However, EO was also tested at 50 ppm to establish a standardized basis of comparison with AgNPs under identical conditions. This approach allowed us to directly evaluate the relative efficacy of both treatments at the same concentration, even though EO at 50 ppm was below its true MIC threshold. Both agents were subsequently assessed for their ability to prevent seed infection in vitro. The results of the in vitro seed germination and radicle growth assay revealed statistically significant differences among the treatments (Table 2 and Figure 8). Seeds treated with AgNPs (50 ppm) exhibited the highest germination rate (100%) and a radicle length of 73.4 mm, suggesting a stimulatory effect on early seedling development. In contrast, seeds exposed solely to R. solani showed a marked decline in germination (45%) and severely stunted radicle growth (18.6 mm), indicating the pathogen's aggressive inhibitory effect. Notably, treatments combining AgNPs or EO with R. solani significantly improved both germination and radicle elongation compared to the pathogen-only group. The control group (distilled water) showed a germination rate of 100% and a radicle length of 65.2 mm. Overall, these findings indicate that AgNPs not only mitigate the negative impact of R. solani but may also enhance early seedling vigor under sterile conditions.

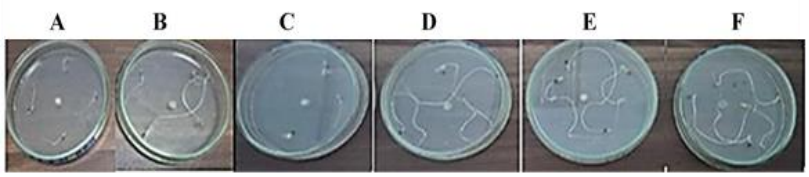

Fig. 8: Effect of *Origanum compactum* Essential Oil (EO) and silver nanoparticles (AgNPs) on tomato seed germination and radicle growth in the presence or absence of *Rhizoctonia solani* (RS). (A) EO + RS, (B) AgNPs + RS, (C) RS only, (D) Control (untreated), (E) EO only, and (F) AgNPs only

Table 2: *In vitro* inhibition of tomato damping-off by AgNPs and EO (50 ppm) based on germination rate (%) and radicle length (mm). Values are expressed as mean ± standard deviation (n = 3)

Treatments	Germination rate (%)	Length of root (mm)
Controle (distilled water)	100.0 ± 0.0	65.2 ± 0.6
AgNPs (50 ppm)	100.0 ± 0.0	73.4 ± 0.7
EO (50 ppm)	100.0 ± 0.0	60.8 ± 1.9
R. solani (RS)	45.0 ± 0.5	18.6 ± 0.3
EO (50 ppm) + *R. solani*	90.0 ± 0.7	39.2 ± 0.5
AgNPs (50 ppm) + *R. solani*	90.0 ± 0.8	45.6 ± 0.2

Inhibition of Tomato Damping-off by AgNPs and EO at 50 ppm Under Greenhouse Conditions

Extended immersion (24 h) of tomato seeds in EO or AgNPs at 50 ppm resulted in total inhibition of germination as shown in Table 3, indicating strong phytotoxicity. In contrast, a 2h immersion yielded optimal protective results without phytotoxic effects, especially with AgNPs, which achieved 86.5% control efficacy against R. solani.

Table 3: Effects of *Origanum compactum* essential oil (50 ppm) and biosynthesized AgNPs (50 ppm) on pre-emergence and post-emergence damping-off of tomato seeds under greenhouse conditions. Values are expressed as mean ± standard deviation (n = 3). Different letters (a–e) within the same column indicate significant differences according to Tukey's test at $p \leq 0.05$. Treatments with 24 h immersion resulted in seed toxicity

Treatments	Immersion time	Damping off pourcentage %			
		Pre-Emergence Damping off (%)	Post-Emergence Damping off (%)	Disease Incidence (DI%)	Control Efficacy (CE%)
Negative Control (Sterile Soil)	—	0.0± 0.0^{e}	0.0± 0.0^{e}	0.0± 0.0^{e}	100%
Pathogen Control (R. solani)	—	48.5± 1.5^{a}	42.0± 1.0^{a}	90.5± 1.2^{a}	0.0%
EO (50 ppm) + R. solani	2h	11.5± 1.2^{b}	6.2± 0.8^{b}	17.7± 1.6^{b}	80.4%
EO (50 ppm) + R. solani	24h	—	—	—	Toxicity
AgNPs (50 ppm) + R. solani	2h	8.2± 1.0^{c}	4.0± 0.6^{c}	12.2± 1.3^{c}	86.5%
AgNPs (50 ppm) + R. solani	24h	—	—	—	Toxicity

Table 4: Effect of AgNPs and EO (50 ppm) on growth parameters of tomato seedlings after 2h immersion

	Growth parameters						
Treatments	**GR (%)**	**PH (cm)**	**RI (cm)**	**SI (cm)**	**VI**	**Fw (g)**	**Dw (g)**
Control (Sterile Soil)	85.0 ± 2.0^{b}	11.2 ± 0.4^{b}	9.8 ± 0.3^{b}	12.2 ± 0.4^{b}	1785	3.90± 0.10^{b}	1.20 ± 0.05^{b}
AgNPs (50 ppm)	92.0 ± 1.5^{b}	12.1 ± 0.5^{a}	10.6 ± 0.3^{a}	13.0 ± 0.3^{a}	2088.04	4.15± 0.08^{a}	1.30 ± 0.04^{a}
EO (50 ppm)	80.0 ± 2.2^{b}	11.0 ± 0.4^{b}	9.4 ± 0.4^{b}	11.8 ± 0.4^{b}	1632	3.85± 0.09^{b}	1.18 ± 0.04^{b}
R. solani (No Treatment)	45.0 ± 0.5^{d}	4.1 ± 0.3^{d}	3.6 ± 0.2^{d}	5.0 ± 0.3^{d}	346.5	1.75± 0.07^{d}	0.55 ± 0.03^{d}
EO (50 ppm) + *R. solani*	70.± 1.8^{c}	9.0 ± 0.3^{c}	7.3 ± 0.2^{c}	9.8 ± 0.3^{c}	1149.15	3.10± 0.08^{c}	1.00 ± 0.04^{c}
AgNPs (50 ppm) + *R. solani*	80.0± 1.5^{b}	9.8 ± 0.4^{b}	8.0 ± 0.3^{b}	10.8± 0.3^{b}	1438.4	3.35± 0.09^{b}	1.08 ± 0.05^{b}

GR (%) = Germination rate, PH (cm) = Plant height (cm), RI (cm) = Root length, SI (cm) = Shoot length, VI = Vigor index, Fw (g) = Fresh weight, Dw (g) = Dry weight

The results obtained from both disease suppression and growth parameter analyses clearly demonstrate the superior performance of green-synthesized silver nanoparticles (AgNPs, 50 ppm) over *Origanum compactum* essential oil (EO, 50 ppm) in promoting tomato seedling development and controlling damping-off caused by *Rhizoctonia solani*. As shown in Table

4, seeds treated with AgNPs alone exhibited a 92 % germination rate, compared to 80% with EO and 85% in the sterile control. AgNPs also significantly enhanced shoot height (12.1 cm), root length (10.6 cm), and seedling vigor index (2088.04), all of which were statistically higher than EO and control treatments ($p < 0.05$).

Under pathogen stress, the AgNPs + *R. solani* treatment (2h immersion) maintained high growth values: 80.0% germination, 9.8 cm shoot height, 8.0 cm root length, and a vigor index of 1438.4, outperforming the EO + *R. solani* treatment (70.5%, 9.0 cm, 7.3 cm, 1149.15, respectively). These differences were statistically significant ($p < 0.05$, Tukey HSD). Disease incidence data confirmed these findings. As shown in Figure 9, AgNPs (2h) reduced total damping-off to 12.2%, significantly lower than EO (17.7%) and much lower than the untreated infected group (90.5%). Furthermore, Figure 10 highlights the superior root development observed in AgNP-treated seeds, especially under *R. solani* infection, where the root systems were visibly denser and more branched compared to pathogen control treatments.

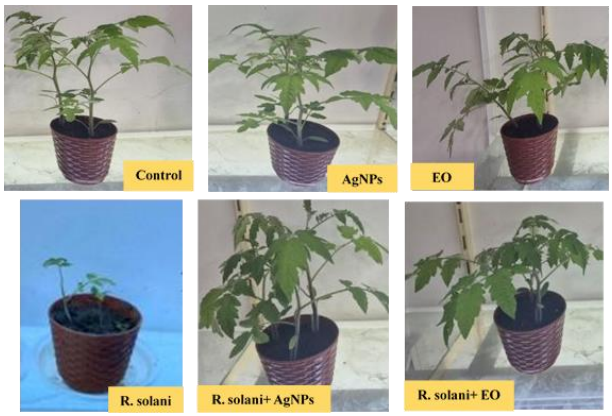

Fig. 9: Damping off by *R. solani* inhibition using EO and AgNPs under greenhouse conditions, tomato seeds treated and untreated with EO and silver nanoparticles at 50ppm after 2h immersion

Fig. 10: The root length of treated and untreated tomato seeds with: Control (1), AgNPs (2), EO (3), *R. solani* (4), *R. solani* + AgNPs (5), *R. solani* + EO (6)

Discussion

The pathogenicity results clearly demonstrated the aggressive nature of *Rhizoctonia solani,* which caused severe pre- and post-emergence damping-off in tomato seedlings both *in vitro* and under greenhouse conditions, with high mortality rates. These findings align with those of [44], who reported rapid wilting, oxidative stress, and structural damage in tomato plants infected with *R. solani*. Multiple studies have identified *Rhizoctonia solani* as one of the most aggressive and damaging soil-borne pathogens impacting tomato production [3, 4]. Its long-term survival in soil is largely attributed to its production of sclerotia durable structures that allow the fungus to persist under adverse conditions. This makes controlling the disease especially challenging in greenhouse environments, where high humidity and temperature levels favor rapid infection and spread. These observations confirm the high virulence of the isolate and underscore the urgent need for environmentally sustainable alternatives to synthetic fungicides. As a result, the development of effective and sustainable biocontrol solutions has become increasingly important.

In this context, the green synthesis of silver nanoparticles using *Origanum compactum* essential oil offers a promising alternative for managing fungal infections. The appearance of a distinct color change and a pronounced surface plasmon resonance peak at 428 nm confirmed the rapid formation of AgNPs, an observation consistent with patterns reported in other plant-based nanoparticle syntheses [45]. This rapid reaction is attributed to the presence of potent bioactive compounds in

the essential oil, such as pulegone, carvacrol, and thymol, which function simultaneously as reducing and stabilizing agents during nanoparticle formation [46].

Characterization results confirmed the formation of silver nanoparticles that were generally spherical and relatively uniform in shape, with most particles measuring between 14 and 20 nm. This nanoscale size range likely contributed to the high antifungal performance observed, as smaller AgNPs possess larger surface area-to-volume ratios, facilitating stronger interactions with fungal membranes. The crystalline structure of the AgNPs was validated by X- Ray Diffraction (XRD), which exhibited distinct peaks corresponding to the face-centered cubic (fcc) phase typical of metallic silver. Raman spectroscopy further indicated successful surface functionalization by bioactive constituents from *Origanum compactum* essential oil, which likely played a role in improving both nanoparticle stability and biological effectiveness [47].

The chemical composition of *Origanum compactum* essential oil used in this study was previously characterized by [27], who identified pulegone (25.79%) and borneol (23.28%) as the dominant constituents, along with notable amounts of α-terpineol (9.86%) and carvacrol (5.79%). These compounds are well-documented for their antimicrobial activity, with carvacrol in particular known to compromise fungal cell membranes and disrupt enzymatic functions. The presence of these bioactive molecules on the surface of silver nanoparticles likely contributes to improved dispersion through steric stabilization, as described by [48], and may also enhance the antifungal performance observed in the present work.

Our *in vitro* results showed that AgNPs and the EO were able to significantly inhibit the mycelial growth of *R. solani* at all concentrations (12.5-150 ppm). The biosynthesized AgNPs exhibited superior antifungal activity, achieving 93.7% inhibition at 50 ppm. Similar effects were observed in several studies. [49] reported that AgNPs at 75 ppm significantly inhibited the mycelial growth of Alternaria alternata and other pathogens responsible for postharvest rot in kiwifruit. Moreover, AgNPs caused severe structural alterations in cellular membranes and organelles, confirming their fungal destruction mechanism through membrane permeabilization. The magnitude of inhibition in our study slightly exceeds those reported previously, which may be explained by the combined presence of phenolic and terpenoid capping molecules from *O. compactum.* At 50 ppm, AgNPs led to a significant reduction in damping-off symptoms also. This finding is consistent with [50], who reported 92.2% inhibition of *R. solani* at similar doses, comparable to the effect of the fungicide Topsin-M. Furthermore [51] demonstrated that even at low concentrations (50–100 µg/L), copper nanoparticles (Cu_2O) effectively reduced root rot in cucumber by activating defense-related enzymes such as catalase and peroxidase.

A key finding of this study was the synergistic effect observed when AgNPs were synthesized with *O. compactum* essential oil. This treatment exhibited stronger antifungal activity than either EO alone, confirming that the bioactive compounds in the essential oil can enhance the interaction between AgNPs and fungal cells, possibly by increasing the permeability of fungal cell membranes to nanoparticles. Similar synergistic effects have been reported in other studies, where the combination of plant extracts and metallic nanoparticles amplified antimicrobial potency, likely due to complementary modes of action [52, 53]. Mechanistically, AgNPs are known to generate Reactive Oxygen Species (ROS) and interfere with membrane integrity, while EO phenolics destabilize lipids and inhibit key fungal enzymes. Their combined action may therefore accelerate cell death and prevent hyphal regeneration. The combined treatment strategy not only enhances antifungal efficacy but also allows for reduced AgNP dosages, which is critical for minimizing the potential phytotoxicity and environmental risks associated with the application of metallic nanoparticles in agriculture [54, 55]. The AgNPs function as carriers, enhancing the stability and dispersion of EOs and facilitating a more uniform and sustained release of the active compounds [56].

However, our results also highlight the importance of optimizing exposure duration during seed treatment to mitigate phytotoxicity. When immersion duration was extended to 24 hours, both AgNPs and EO treatments showed signs of toxicity, such as inhibition of germination and growth. In contrast, shorter immersion times of 2 hours maintained high disease control efficacy without compromising seed viability. These findings underline the need for precise application protocols when using nanoparticles in agriculture.

On the other hand, while several studies (including our own) have shown that AgNPs can enhance plant growth, increasing attention is being paid to their potential toxicity and concentration [55]. Silver nanoparticles may interfere with critical physiological processes such as chlorophyll function, photosynthetic efficiency, nutrient uptake, hormone regulation, and transpiration. These disruptions could ultimately compromise plant health and productivity if not properly managed [57, 58]. Therefore, before large-scale implementation, it is crucial to assess nanoparticle risks and determine safe concentrations to ensure both effectiveness and safety.

The seed treatment assays under both *in vitro* and greenhouse conditions demonstrated that the AgNPs not only reduced pre- and post-emergence damping-off but also enhanced germination rates, root elongation, and seedling vigor (2088.4 compared to control 1785). These results suggest that in addition to its direct antifungal activity, the treatment may exert indirect plant growth-promoting effects, possibly through improved root health and enhanced nutrient uptake resulting from healthier root systems. The findings of this study are consistent with those of [59], who demonstrated that silver nanoparticles synthesized using Eucalyptus camaldulensis leaf extract significantly enhanced germination, growth, biomass, and resistance to *Rhizoctonia solani* in Acacia mellifera and Acacia senegal seedlings.

This dual effect disease suppression combined with enhanced plant vigor has been reported for other biosynthesized nanoparticles, particularly when they are stabilized by bioactive plant extracts rich in secondary metabolites with known plant growth-promoting properties [21, 60]. Thus, the AgNP–EO system may contribute both to pathogen inhibition and to improved physiological performance in tomato seedlings.

The use of biosynthesized silver nanoparticles (AgNPs) combined with essential oil in tomato seed treatments presents a viable and sustainable alternative to conventional fungicides, which face growing concerns due to their environmental impact and potential health risks. The green synthesis approach (based on abundant plant materials and free of toxic chemicals) adds to the ecological advantages of this method [25].

While essential oils alone demonstrate antifungal activity, their practical use in greenhouse and field conditions is limited by volatility, reduced persistence, sensitivity to light and temperature, and the need for higher application concentrations, which increases cost and limits scalability. In contrast, AgNPs provide a more stable, long lasting, and cost-effective solution that maintains efficacy at lower doses, making them a more viable and efficient tool for integrated disease management strategies.

Despite these promising results, it is important to highlight potential environmental concerns related to the large-scale use of silver nanoparticles. Several studies have reported that AgNPs may accumulate in soil and interact with non-target microorganisms, potentially altering microbial community structure, nutrient cycling, and soil fertility [61]. Moreover, the persistence and transformation of AgNPs in natural environments remain poorly understood, raising questions about their long-term ecological impact. Therefore, future research should not only focus on optimizing antifungal efficacy but also assess the biodegradability, mobility, and biosafety of biosynthesized AgNPs under realistic field conditions. Addressing these aspects will be crucial to ensure the safe integration of nanotechnology into sustainable agricultural practices.

Conclusion

In summary, silver nanoparticles synthesized via a green method using *Origanum compactum* essential oil represent an eco-conscious and efficient strategy for managing *Rhizoctonia solani,* a key soil-borne pathogen affecting tomato crops. The combination of AgNPs with essential oil displayed strong synergistic antifungal effects, effectively lowering both pre- and post-emergence damping-off rates under laboratory and greenhouse settings.

This integrated approach takes advantage of the unique physicochemical traits of nanoparticles and the bioactive components of the essential oil. It offers a viable, environmentally safe substitute for conventional fungicides. Continued investigation is necessary to evaluate field- scale effectiveness, safety profiles, and economic feasibility. Ultimately, this biocontrol method supports the transition toward sustainable agriculture and aligns with global objectives such as the United Nations Sustainable Development Goals (specifically SDG 2 and SDG 12).

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Author's Contributions

Laila Reklaoui: Conceptualization, experimental investigation, data curation, original draft writing.
Zine El Abidine Bzazou Elouazzani: Silver nanoparticle synthesis, and methodology optimization.
El Touhami Tayebi Ouazzani: Contribution to fungal culture preparation; support in greenhouse trials.
Ferdaous Dmini and Mariam El Idrissi El Berkani: Contribution to fungal culture preparation; support in greenhouse trials.
Abderazzak Rfaki and Hassan Ghazal: Data analysis, statistical validation.
Haiat Essalmani: Scientific coordination, critical revision and results interpretation.
Said Barrijal: Project administration, overall supervision, and final manuscript approval.

Ethics

This study involved only *in vitro* and greenhouse-based experiments on plants and did not require ethical approval.

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