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Management of onion white rot caused by *Sclerotium cepivorum* Berk using bio-synthesized silver nanoparticles

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ABSTRACT

The onion (*Allium cepa* L.) stands as a significant vegetable crop globally, including Egypt. However, its susceptibility to white rot, a severe disease caused by the soil-borne fungus *Sclerotium cepivorum*, poses a substantial threat to *Allium* spp. In our study, we illustrate a bio-based approach to synthesizing silver nanoparticles (Ag-NPs) using neem extraction (*Azadirachta indica*) leaves and *Trichoderma reesei* (formerly known as *Hypocrea jecorina*). The reaction process is monitored using UV-visible light spectroscopy, while fluorescence emission spectroscopy provides detailed insights into the nanosecond time frame of Ag-NP creation. Additionally, Fourier transforms infrared spectroscopy (FTIR) aids in quantitatively analyzing the reaction products. Throughout the reaction period, the surface Plasmon band in the silver nanoparticle solution remains consistently near 429 nm. Furthermore, measurements reveal an average size distribution (DLS) of 66.2 nm for the Ag-NPs, with those synthesized from neem leaf extract exhibiting a negative zeta potential of -22.93mv.In vitro experiments demonstrate the inhibitory effect of *T. reesei*-produced silver nanoparticles (Ag-NPs-Tr) and neem extract-derived nanoparticles (Ag-NPs-Ne) on *S. cepivorum* mycelial growth at a concentration of 250 µL/L. However, it is noteworthy that the *T. reesei* strain used in this study displayed tolerance to Ag-NPs at all tested doses. Moreover, the application of Ag-NPs-Tr and Ag-NPs-Ne, or fungicides, via dipping onion transplants led to a reduction in onion white rot disease incidence. Notably, Ag-NPs-Tr at a concentration of 250 µL/L exhibited similar efficacy to Flumid 24%, resulting in the most significant reduction in onion white rot incidence (11.1%) and increased onion bulb yield (256.3 and 258.8g/pot, respectively).In conclusion, our findings suggest that biosynthesized silver nanoparticles offer promising potential in managing onion white rot disease.

Key words: Extracellular biosynthesis; Silver nanoparticles; *Trichoderma reesei*; Neem extract; *Sclerotium cepivorum*; Flumid 24% fungicide.

INTRODUCTION

Globally, as well as in Egypt, onions (*Allium cepa* L.) hold a crucial position as a staple vegetable crop. The menace of white rot, induced by the soil-borne fungus *Sclerotium cepivorum*, poses a significant

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threat to *Allium* species. This fungus has the capability to persist in the soil for extended periods, spanning up to twelve or even fifteen years (Pung *et al.,* 2008). Chemical fungicides such as folicur have traditionally been the primary recourse for disease management. In Egypt, the scourge of white rot has decimated allium farms, resulting in devastating losses.

The symptoms of white rot manifest as wilting, yellowing of older leaves, and die-back of leaf tips, primarily evident above ground (Abd-Elrazik *et al.,* 1973). This progression eventually leads to the collapse and decomposition of leaf blades, facilitated by the spread of symptoms along the blades. Root infection gives rise to watery rot at the base of the bulbs, culminating in the formation of fluffy, white mycelial growth mats. These mycelial mats harbor thousands of sclerotia, serving as potential sources of infection for subsequent crops of *Allium* spp. Unmarketable bulbs are a common consequence of tissue colonization in garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) plants (Ulacio-Osorio *et al.,* 2006).Unlike many pathogens, *Sclerotium cepivorum* does not produce spores in its natural state; instead, it persists on diseased portions of the host plant and survives in the form of sclerotia, typically ranging from 0.3 to 0.6 mm in diameter (Brix &Zinkernagel, 1992). These sclerotia exhibit remarkable durability; with the ability to endure in the soil for nearly 30 years in the absence of suitable hosts (Crowe *et al.,* 1993).A mere handful of sclerotia can initiate disease development (Crowe *et al.,* 1980). To safeguard Allium plants against this infection, a sequential application of non-environmental chemical fungicides, such as vinclozolin, tebuconazole, and triazole, has been employed. However, the efficacy of these fungicides often diminishes over time due to microbial degradation in the soil (Entwistle & Hawling, 1984; Jackson *et al.,* 1997). Consequently, soil fumigation with fungicides has been explored as a disease management approach (Merriman & Sutherland, 1978; Adams & Johnston, 1983). Yet, the utilization of certain agents like methyl bromide has dwindled due to their adverse effects on the environment and human health. Hence, safer alternatives are sought after. Bio-fungicides, including sclerotial mycoparasites, antagonistic bacteria, and Trichoderma species, have emerged as promising alternatives for combating white rot disease (Elshahawy *et al.,* 2017a, 2017b, 2018). An effective strategy involves reducing early inoculum density in the soil through the use of sclerotia germination stimulants (Elshahawy *et al.,* 2019, 2020). However, the efficacy of these treatments is contingent upon the density of live *S. cepivorum* sclerotia (Elshahawy *et al.,* 2018). The overuse of chemical fungicides poses significant environmental and health concerns, prompting the exploration of fungicide substitutes. The advent of nanotechnology has rendered the production of nano-sized silver particles more economical, thus making them viable antimicrobial agents. Safer and more efficient alternatives to fungicides are urgently needed, considering the emergence of fungal infections resistant to conventional treatments, and the associated health and environmental risks. Nanotechnology, which involves the manipulation of size and shape at the nanoscale, offers a promising avenue for designing, characterizing, manufacturing, and utilizing structures, devices, and systems (Mansoori, 2005). Various techniques can be employed to prepare nanoparticles from a wide range of materials, utilizing precursors from liquid, solid, or gas phases (Mansoori, 2005). The demand for environmentally friendly nanoparticles in synthesis protocols that minimize hazardous waste generation is on the rise. Furthermore, nanobiotechnology enables

the development of reliable procedures for chemical composition and synthesis, leveraging biological entities such as microorganisms and living cells, which possess nanoscale operating components capable of efficiently performing diverse tasks (Goodsell, 2004).

The utilization of microorganisms such as bacteria, fungi, yeasts, and herbal extracts to synthesize nanoparticles represents a novel approach crucial for remediation of toxic metals through metal ion reduction. These benign nanofactories offer ecologically friendly biological systems, provided they pose no other toxic risks. Biological synthesis examples include plant extracts (Huq *et al.,* 2022), microorganisms (Lobregas and Camacho, 2022), and agricultural wastes (Saha and Kim, 2022). Plant extracts contain bio-chemicals like phenolic compounds, alkaloids, terpenoids, sugars, enzymes, and proteins, which convert metallic salts from a positive to a zero oxidation state (Sudheer *et al.,* 2022). Moreover, these extracts serve as capping agents during Ag-NPs production, preventing nanoparticle aggregation, reducing toxicity, and enhancing antibacterial activity (Ahmed *et al.,* 2016). In this study, we explored the synthesis of Ag-NPs using *Trichoderma reesei* and *Azadirachta indica* (neem) leaf extract. *T. reesei*, a filamentous fungus thriving in decomposing plant matter, is widely utilized in industry for its high production of plant cell wall-degrading enzymes and performance proteins. Neem, a member of the Meliaceae family, contains polyphenolic flavonoids like quercetin and β-sitosterol in its leaves, exhibiting antifungal and antibacterial properties (Srivastava *et al.,* 2020). Neem leaves also boast anti-inflammatory, immunomodulatory, antiulcer, anti-hyperglycemic, antioxidant, anti-mutagenic, anti-carcinogenic, antimalarial, antifungal, antibacterial, and antiviral properties (Girish and Shankara, 2008). Given neem leaves' natural antibacterial nature, their antimicrobial efficacy is enhanced through Ag-NPs synthesis, with neem leaf extract serving as the reducing and stabilizing agent (Ahmed *et al.,* 2016). Biosynthesis of Ag-NPs using microbial and plant agents has gained traction due to their controlled and ecologically benign nature (Marrez *et al.,* 2019; Rehman *et al.,* 2019, 2020a, 2020b). One promising application of Ag-NPs is plant disease control (Jo *et al.,* 2009; Min *et al.,* 2009). Ag-NPs exhibit inhibitory effects on bacteria, making them safer and more effective alternatives to fungicides when applied to plants (Park *et al.,* 2006; Kim *et al.,* 2012). The current study aims to synthesize silver nanoparticles using neem leaf extract and *Trichoderma reesei* and evaluate their efficacy in suppressing *Sclerotium cepivorum*, the causal agent of onion plant white rot disease.

MATERIALS AND METHODS

Source of bioagent:

The fungal species *Trichoderma reesei* isolate (AUMC5829) utilized in this study was acquired from the Plant Pathology Department, Faculty of Agriculture, Mansoura University.

Source of the pathogen:

A pathogenic *Sclerotium cepivorum* isolate was sourced from Dr. Mamdouh M. Abdel-fattah Khalifa, Senior Researcher at the Plant Pathology Institute, Agricultural Research Center (ARC) in Giza, Egypt.

Biosynthesis of silver nanoparticles using Trichoderma reesei (AUMC5829):

Preparation of biomass:

In order to synthesize silver nanoparticles, 200 mL containers containing 100 mL of potato dextrose broth medium (PDB) were cultured for 72 hours at a temperature range of 25–26°C with a variation of ±2°C on a rotary

shaker running at 150 revolutions per minute (rpm). After extracting the mycelial (vegetative portion of the fungus) mass with sterile filter paper from the culture broth, the biomass was thoroughly cleaned using three rinses with sterile distilled water (Whatman No. 1). The mycelial material that was removed was then used to make silver nanoparticles.

Biosynthesis of silver nanoparticles using *Trichoderma reesei* (Ag-NPs-Tr):

Twenty-five-gram mycelial mats were placed in flasks with one hundred milliliters of distilled water that had been sterilized, and the mixture was incubated at twenty-five degrees Celsius for a full day. The unbound crude filtrate was collected for further analysis after biomass refining. A 250-mL Erlenmeyer flask was used to biosynthesize silver nanoparticles (Ag-NPs). The process involved combining 10 mL of cell filtrate with 90 mL of a 1 mM AgNo3 solution and then letting the combination sit at 28°C for 72 hours under dark conditions. Simultaneously, a control flask with cell-free filtrate but no silver nitrate solution was used as a standard. For more experimentation, this solution acted as the stock solution.

Biosynthesis of silver nanoparticles using neem leaf extracts (AgNPs-Ne):

Preparation of extract from neem (*Azadirachta indica*):

Freshly matured Neem (*Azadirachta indica*) leaves were sourced from Fac. Agric. Moshtohor. These leaves underwent a meticulous preparation process, including two washes in tap water followed by three rinses in distilled water. Subsequently, the leaves were air-dried for three days at room temperature (approximately 25°C) and then ground into powder over the course of three days using an electronic blender. To obtain an extract, 100 mL of deionized water and 5 g of leaf powder were boiled for 30 minutes in a 500 mL conical flask. After cooling, the extract was filtered through Whitman No. 1 filter paper into a sanitized, dry beaker. The filtered extract was then stored at 4°C until required for further use.

Biosynthesis of silver nanoparticles from neem leaf extract:

The process of biosynthesizing silver nanoparticles was observed within a dark brownish-yellow solution, resulting from the combination of 10 mL of neem filtrate with 90 mL of 1 mM silver nitrate (AgNO3) aqueous solution. This mixture, prepared by dissolving 21.2 g of AgNO3 powder in 125 mL of Milli Q water, was incubated with an orbital shaker operating at 120 rpm/min at room temperature for approximately 3 hours. This method aligns with the procedure described by Gnanadesigan *et al.,* (2011), and the aforementioned stock solution was utilized throughout the experiment.

Characterization of silver nano-particles:

UV-visible spectroscopy analysis:

Upon incubation with the silver nitrate solution, a noticeable alteration in the color of the cell-free filtrate was observed. The bio-reduction process of silver ions was assessed by extracting aliquots of 1 mL at various time intervals. The evaluation of absorption was conducted using a UV-visible spectrophotometer (LW-200 Series), with absorbance measurements taken within the wavelength range of 200 to 800 nm.

Dynamic light scattering (DLS) and Zeta Potential Analysis:

Dynamic light scattering, employing the laser diffraction principle and encompassing various scattering techniques, was utilized to analyze the average particle size of the silver nanoparticles. The prepared sample

underwent separation via ultrasound following dilution in deionized water. Subsequently, the mixture was subjected to filtration and centrifugation at 25°C and 5000 rpm for 15 minutes, yielding a supernatant. This supernatant was then collected and further diluted four to five times before examination in a computer-controlled particle size analyzer (Zeta sizer Nano, Malvern Instruments Ltd., UK).

Transmission electron microscopy (TEM):

The size and morphology of the produced nanoparticles were investigated using a JEM-1200 EX electron microscope manufactured by JEOL, Japan. Thin films of the sample were prepared by dropping a minute amount onto a carbon-coated copper grid. Excess solution was carefully removed by blotting with filter paper, and the film was subsequently dried on the TEM grid within an incubator. This process facilitated the examination of the nanoparticles' characteristics under the electron microscope.

Effect of silver nanoparticles (AgNPs) synthesized by *T. reesei* (AgNPsTr) and neem leaf extract (AgNPs-Ne) on the linear growth of *Sclerotium cepivorum in vitro*:

The purpose of this investigation was to investigate if two Ag-NPs generated by *T. reesei* and neem extract could suppress the linear growth of *S. cepivorum in vitro*. The Ag-NPs were evaluated under various concentrations, including 125, 250, 500, 1000, 2000, 4000, and 8000 µL /L. The inhibitory effect of biogenic Ag-NPs on the linear development of *S. cepivorum in vitro* was investigated using the pour plate method (Min *et al.,* 2009). In this regard, the Ag-NPs were sterilized using a Millipore filter before being introduced to the warmed 100 mL PDA medium. PDA medium was gently mixed in each conical flask before being poured in a constant amount (15 mL) into sterilized Petri dishes (9cmØ) and allowed to harden. The medium containing no Ag-NPs acted as a control. 5mm mycelium discs from the edge of 10-day-old fungal cultures placed in the center of Petri dishes. For each concentration as well as the control treatment, three plates were employed. Plates were incubated at 18±2ºC in an incubator. When mycelium covered the medium surface in the control treatment, the experiment was called off. Averaging the two diameters collected at right angles for each colony was used to calculate fungal growth. After incubation, the growth inhibition percentage (GI %) for each treatment was calculated according to Arora and Upadhay (1978) as follows: GI%= ((C-T) /C) *100 where, GI% = percent of growth inhibition over control, C = radius growth of control (mm), T = radius growth of treatment (mm).

Greenhouse Experiments:

Following thorough mixing with a 5% commercial formalin solution (one liter of formalin solution per cubic foot of soil mixture), loamy sand soil (with a composition of 3 parts clay to 1 parts and by weight) underwent sterilization and was covered with polyethylene for a duration of two weeks. Subsequently, the soil was raked daily for ten days to facilitate formalin evaporation and ensure aeration once the plastic cover was removed.

Effect of AgNPs synthesized by *T. reesei* and neem leaf extract as a dipping treatment on controlling white rot disease of onion:

This study evaluated neem extract, *T. reesei* AgNPs, and neem extract, *T. reesei* without silver nitrate as dipping treatments for controlling white rot disease of onion under greenhouse conditions compared with fungicides. The tested treatments were:

A. Neem Ag NPs and neem without AgNPs at concentrations 125, 250, and 500 µL /L

B. *T. reesei* AgNPs and *T. reesei* without AgNPs at 125, 250, and 500 µL/L concentrations.

C. Fungicides Flumid 24% (2',6'-Dibromo-2-methyl-4'-trifluoromethoxy-4-trifluoromethyl-1,3- thiazole- 5-carboxanilide) and Celest FS 10% (4-(2,2-Difluorobenzo[d][1,3] dioxol-4-yl)-1H-pyrrole-3-carbonitrile) (Syngenta Agro, Egypt) were used in the greenhouse for comparison. The chemical treatments were applied at the recommended rate of 80mL Flumid /100 kg and 1.5cm3Celest/kg. 45-day-old, healthy onion transplants (Giza 20 cv.) were dipped before being planted in each treatment for two hours. Subsequently, at 30, 60, and 90 days after planting, the plant stem bases were sprayed three times with the same concentrations. In every soil container that was infected, transplants of onions were placed. As a control treatment, only water-dipped transplants were utilized. Each pot held three transplants, and three pots were utilized as replicates for each treatment.

Disease assessment:

The disease evaluation was based on the percentages of disease incidence (DI) at harvest, 100 days following planting. It was computed using the formula below, per Hovius and Goldman (2004):

$$\text{White rot infection \%} = \left(\frac{\text{No.of transplant infected with white rot}}{\text{Total No.of plants}}\right) \times 100$$

Data collection:

Following 100 days post-transplanting, the growth parameters of onion plants were assessed, encompassing measurements such as plant height (in centimeters), in both the control and treatment groups. Additionally, the onion bulbs from each pot were carefully removed and weighed to ascertain the yield achieved under different experimental conditions. These measurements provided valuable insights into the impact of the treatment on the growth and productivity of onion plants.

Statistical analyses:

All conducted studies underwent statistical analysis employing the methods described by Snedecor and Cochran (1989), specifically utilizing analysis of variance (ANOVA). Subsequently, the least significant difference test (LSD) was employed to compare treatment means at a 5% probability level. This statistical approach allowed for robust examination and comparison of the experimental treatments, ensuring the validity and reliability of the obtained results.

RESULTS

Synthesis of silver nanoparticles by *T. reesei* (AgNPs-Tr):

After a 72-hour dark incubation period, the culture filtrate containing silver nitrate salt exhibited a deep brown hue. This observation contrasts with the control group, depicted in Figure 1, where no significant color change occurred. The solution containing the fungal filtrate displayed a distinct yellowish-brown tint, indicative of the formation of silver nanoparticles within the reaction mixture. This alteration in coloration can be attributed to the excitation of surface plasmon vibrations of silver nanoparticles, which essentially represent the oscillation of group conduction electrons, thereby imparting the solution its characteristic color.

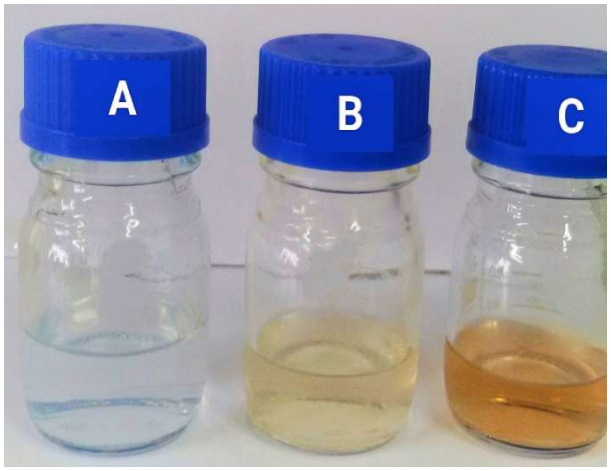

Fig.1: Biosynthesis of Ag-NPs using *T. reesei*: (A) bottles containing silver nitrate solution, (B) Crude cell filtrate of *T. reesei* before mixing with $AgNO_3$, (C) Silver nanoparticles solution.

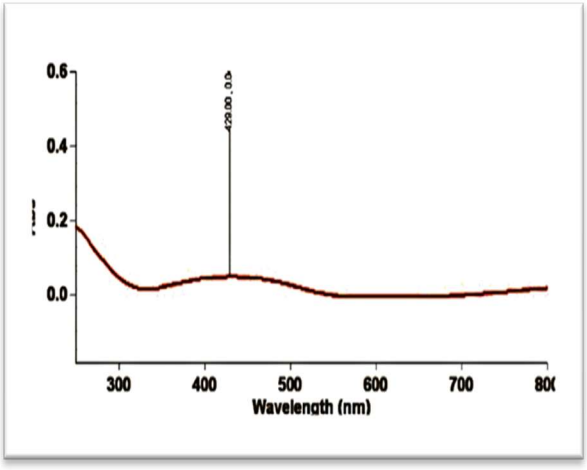

Fig.2. UV-Vis spectra of silver nanoparticles synthesized by *T. reesei*.

Characterization of AgNPs:

UV-Vis Spectrophotometer

UV-Vis spectroscopy serves as a straightforward method to monitor the formation of metal nanoparticles resulting from *T. reesei* exposure, facilitated by the reduction of aqueous metal ions. Figure 2 illustrates the UV-Vis absorption spectrum of silver nanoparticles in the presence of *T. reesei*. Notably, the Surface Plasmon band observed in the silver nanoparticle solution remains consistently near 429 nm throughout the incubation reaction period. This stability suggests that the nanoparticles were uniformly distributed throughout the aqueous solution, indicative of a controlled and efficient synthesis process.

Dynamic Light Scattering (DLS) and Zeta Potential Analysis:

The size distribution profile of nanoparticles in suspension was determined utilizing the Dynamic Light Scattering technique with the Zeta Sizer (Malvern). The average size distribution of Ag-NPs was found to be 89.3 nm, as depicted in Figure 3. Additionally, Ag-NPs biosynthesized from *T. reesei* exhibited a negative zeta potential of approximately -34.11 mV.

TEM analysis of silver nano-particles:

Transmission electron microscopy (TEM) analysis was utilized to discern the size, shape, and morphology of silver nanoparticles. The TEM data revealed that the synthesized silver nanoparticles predominantly exhibit a spherical shape and are well dispersed. However, some of these nanoparticles display irregularly shaped structures, as illustrated in Fig.4

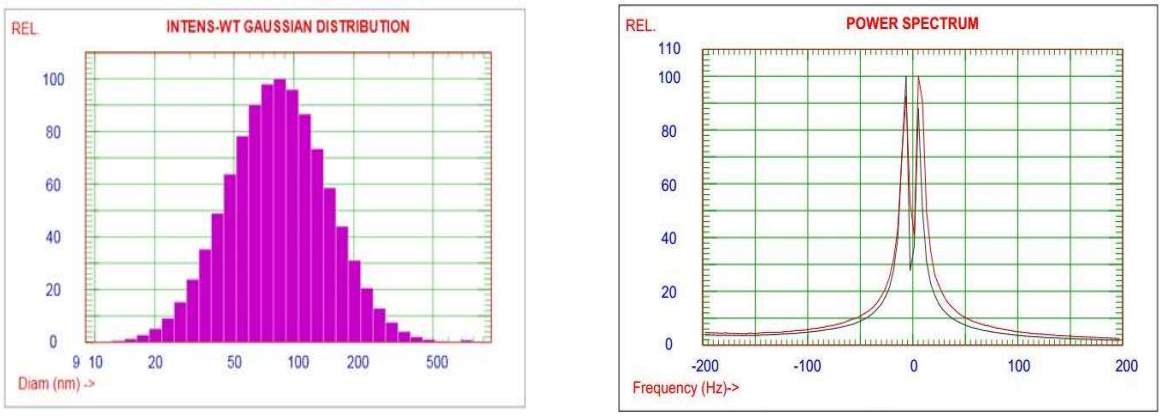

Fig.3: Characterization of the biosynthesized Ag-NPs by the *T. reesei* (F1) Particle size distribution analysis, (F2) Zeta potential measurements of the biosynthesized Ag-NPs.

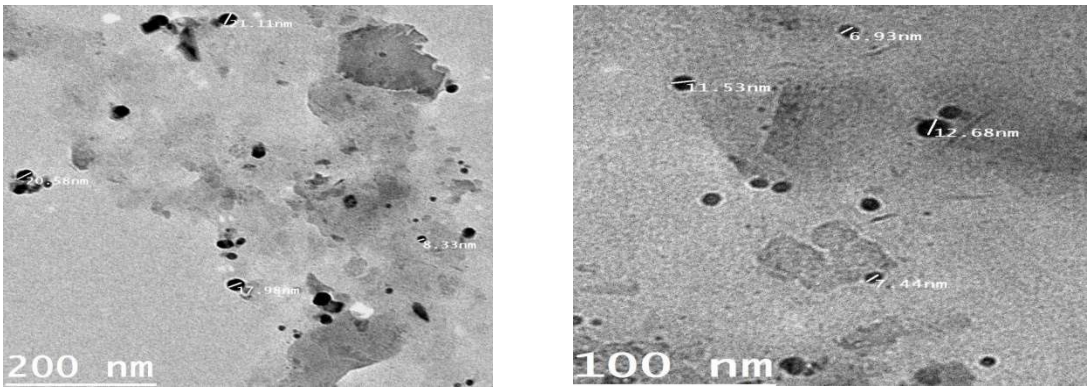

Fig. 4. TEM micrographs of silver nanoparticles synthesized from *T. reesei.*

Syntheses of silver nanoparticles by neem extract (Ag-NPs-Ne):

The biosynthesis of silver nanoparticles from neem extract was successfully achieved, yielding Ag-NPs. Upon challenging the plant extract, a noticeable color shift was observed in the AgNO3 solution, providing initial confirmation of Ag-NP production. Specifically, the mixture of AgNO3 and the aqueous extract rapidly transitioned to various shades of brown, indicative of nanoparticle formation. In contrast, the AgNO3 solution devoid of plant extract remained unchanged, as depicted in Figure 5.

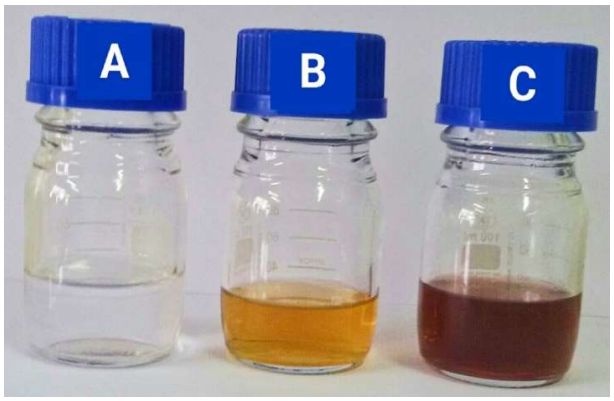

Fig. 5: Biosynthesis of AgNPs using aqueous neem leaf extract: (A) bottles containing silver nitrate solution, (B) plant extract, and (C) the AgNPs solution.

Characterization of Ag-NPs:

UV-Vis Spectrophotometry

The synthesis of metal nanoparticles can be effectively monitored using UV-Vis spectroscopy, particularly through the reduction of aqueous metal ions in the presence of neem extract. Figure 6 illustrates the UV-Vis absorption spectrum of silver nanoparticles in the presence of neem extract. Notably, the Surface Plasmon band of the silver nanoparticle solution consistently remains near 478 nm throughout the reaction period. This observation suggests that the nanoparticles were uniformly distributed throughout the aqueous solution and that there was no evidence of aggregation, as indicated by the absence of significant changes in the UV-Vis absorption spectra.

Dynamic Light Scattering (DLS) and Zeta Potential Analysis:

Figure 7 provides a visualization of the average size distribution (DLS) of Ag-NPs, which measured 66.2 nm. Notably, Ag-NPs synthesized from neem extract demonstrated a negative zeta potential of approximately -22.93 mV, as depicted in Figure 6.

TEM analysis of silver nanoparticles

The size, shape, and morphology of nanoparticles were comprehensively evaluated using transmission electron microscopy (TEM). While some of the silver nanoparticles exhibited irregularly shaped structures, as depicted in Fig. 8, the microscopy analysis revealed that the majority of the particles were spherical and well-dispersed.

Silver nanoparticles (Ag-NPs) were synthesized with or without the utilization of filtrates from *T. reesei* or neem extract, cultivated in the presence or absence of silver nitrate, as confirmed through physicochemical examination. The alteration in color of the filtrates from light yellow to reddish-brown, observed seventy-two hours after the addition of AgNO3, can be attributed to the surface plasmon resonance of silver, as described by Elamawi *et al.,* (2018).Moreover, the separation of silver nanoparticles observed in the TEM image could be attributed to protein capping, which is further supported by the well-dispersed nature of the nanoparticles as indicated by the results of UV-Vis spectroscopy. Additionally, the crystalline nature of the silver nanoparticles is demonstrated by the selected area diffraction pattern (Figure 8), acquired from one of the nanoparticles within the aggregates.

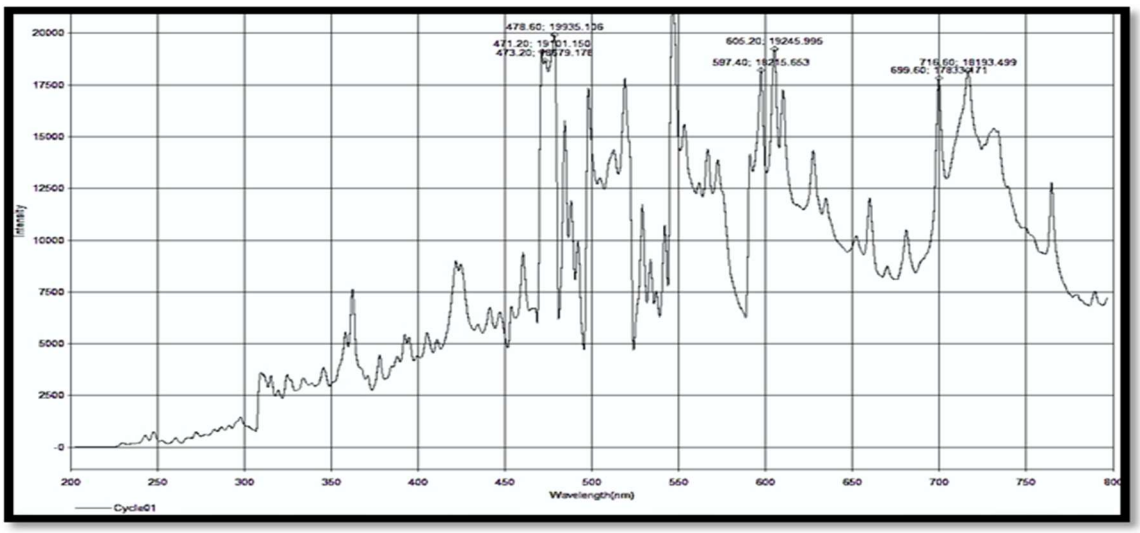

Fig.6. UV-Vis spectra of silver nanoparticles synthesized by neem extract.

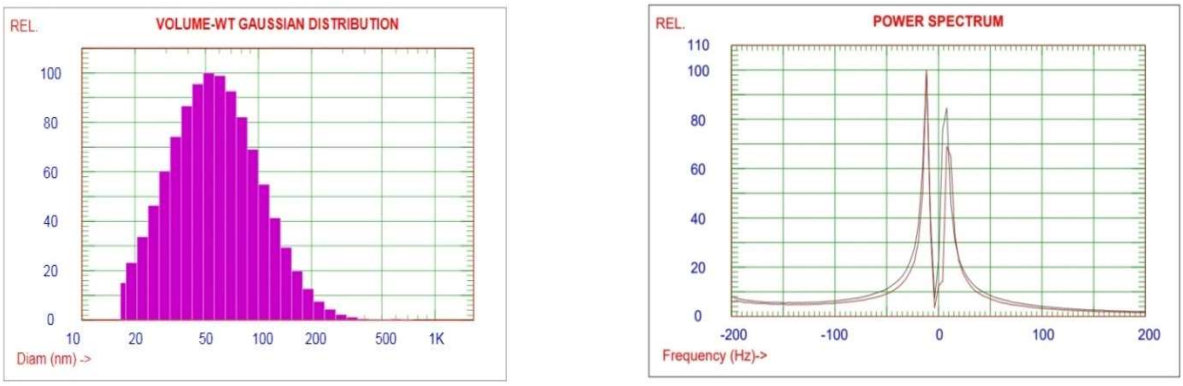

Fig.7: Characterization of the biosynthesized AgNPsbyneem extract: (F1) Particle size distribution analysis, (F2) Zeta potential measurements of the biosynthesized AgNPs.

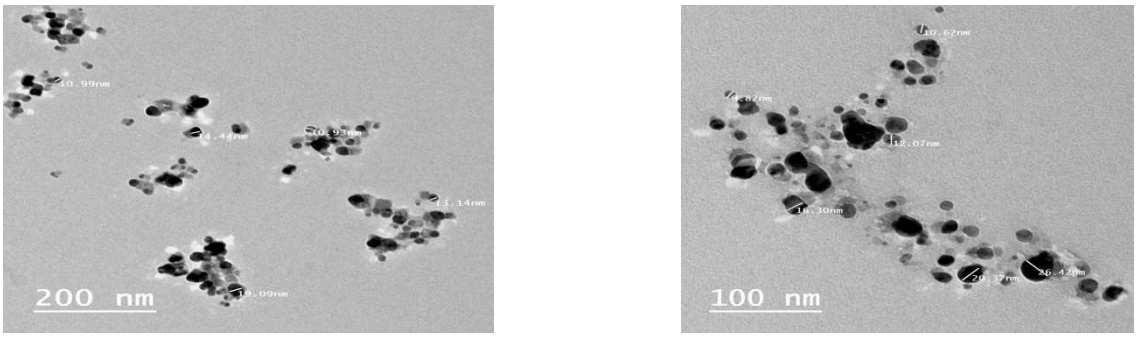

Fig. 8. TEM micrographs of silver nanoparticles synthesized from neem leaf extract.

Effect of silver nanoparticles (AgNPs) synthesized by *T. reesei* on the growth of *Sclerotium cepivorum* and *T. reesei in vitro*:

Compared to the control group, all tested Ag-NPs exhibited inhibitory effects on *Sclerotium cepivorum* development, as demonstrated in Table 1 and illustrated in Fig. 9. The decline in linear growth increased proportionally with the concentration of the tested treatments. Specifically, treatment with 8000 and 4000 µL/L dosages of *T. reesei* Ag-NPs resulted in a reduction of *S. cepivorum* growth by 85.2% and 62.6%, respectively. It is noteworthy that high concentrations of Ag-NPs notably impacted mycelial growth, as evidenced by the reduction in colony diameter. However, *T. reesei* demonstrated tolerance to Ag-NPs at all concentrations investigated in our study.

Effect of silver nanoparticles (Ag-NPs) synthesized by neem leaf extract on growth of *Sclerotium cepivorum* and *T. reesei in vitro*:

To assess the potential of nanoparticles in regulating the mycelial development of *Sclerotium cepivorum*, doses of Ag-NPs from neem leaf extract were administered at concentrations ranging from 125 to 8000 µL/L. As illustrated in Table 2 and Figure 10, the results revealed that, compared to the control group, every tested Ag-NPs from neem extract exhibited a reduction in the growth of *S. cepivorum*. This decrease in linear growth was further pronounced with an increase in the concentration of the examined treatments. Specifically, treatment with neem Ag-NPs at doses of 8000 and 4000 µL/L resulted in a reduction of *S. cepivorum* growth by 87.8% and 74.4%, respectively. These findings suggest the potential efficacy of neem Ag-NPs in inhibiting the mycelial development of *S. cepivorum*, indicating their promise as antifungal agents.

Table 1: Effect of silver nanoparticles (Ag-NPs) synthesized by *T. reesei* on the growth of *Sclerotium cepivorum* and *T. reesei in vitro.*

Treatment	Conc. µL/L	Mycelium growth of *S. cepivorum* (1)	Mycelium growth of *T. reesei* (2)	% Efficacy (1)	% Efficacy (2)
***T. reesei* Ag-NPs**	**125**	75.0	90.0	16.67	0.0
	250	71.7	90.0	20.4	0.0
	500	60.0	90.0	33.3	0.0
	1000	53.3	90.0	40.7	0.0
	2000	39.3	90.0	56.3	0.0
	4000	33.7	90.0	62.6	0.0
	8000	13.3	90.0	85.2	0.0
***T. reesei* without silver nitrate**	**125**	86.0	90.0	4.4	0.0
	250	80.3	90.0	10.7	0.0
	500	71.0	90.0	21.1	0.0
	1000	60.7	90.0	32.6	0.0
	2000	54.3	90.0	39.6	0.0
	4000	41.7	90.0	53.7	0.0
	8000	33.0	90.0	63.3	0.0
Control		90.0	90.0	0.0	0.0
LSD at 0.05		**2.23**			

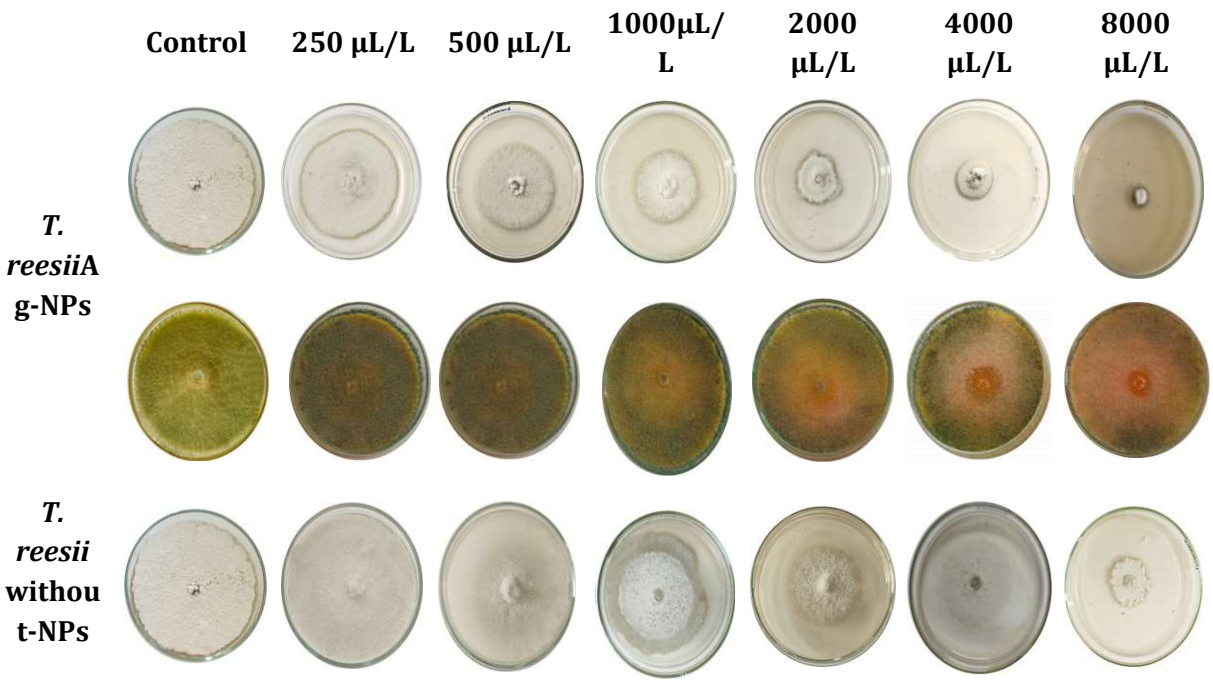

Fig. 9: Effect of silver nanoparticles (Ag-NPs) synthesized by *T. reesei* on the growth of *Sclerotium cepivorum* and *T. reesei in vitro.*

Table 2: Effect of silver nanoparticles (Ag-NPs) synthesized by neem leaf extract on the growth of *Sclerotium cepivorum* and *T. reesei in vitro*.

Treatment	Conc. µL/L	Mycelium growth of *S. cepivorum* (1)	Mycelium growth of *T. reesei* (2)	% Efficacy (1)	% Efficacy (2)
Neem extract AgNPs	125	76.0	90.0	15.6	0.0
	250	73.0	90.0	18.9	0.0
	500	59.0	90.0	34.4	0.0
	1000	44.6	90.0	50.4	0.0
	2000	35.0	90.0	61.1	0.0
	4000	23.0	90.0	74.4	0.0
	8000	11.0	90.0	87.8	0.0
Neem extract without silver nitrate	125	90.0	90.0	0.0	0.0
	250	88.0	90.0	2.2	0.0
	500	80.0	90.0	11.1	0.0
	1000	71.7	90.0	20.4	0.0
	2000	63.0	90.0	30.0	0.0
	4000	52.7	90.0	41.5	0.0
	8000	35.3	90.0	60.7	0.0
Control		90.0	90.0	0.0	0.0
LSD at 0.05		1.86			

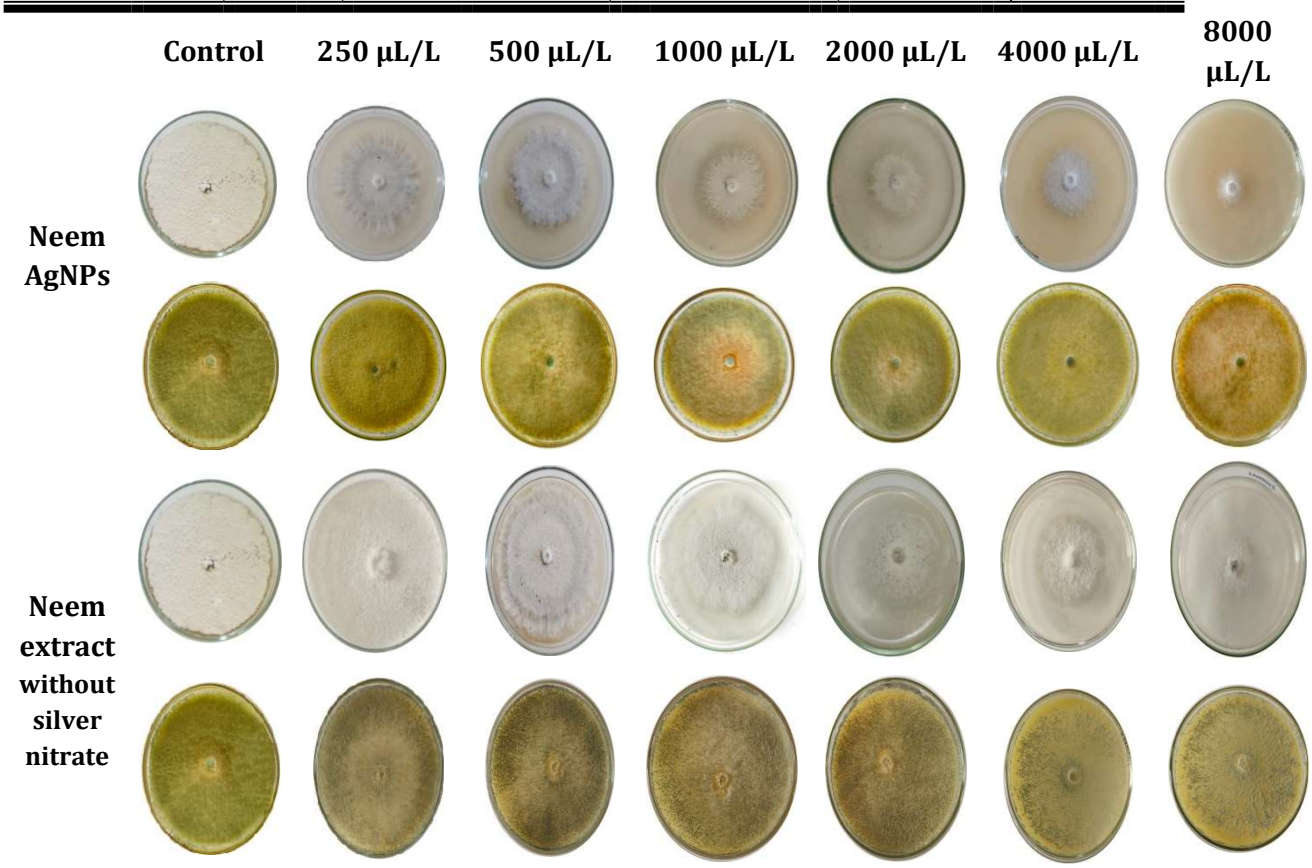

Fig. 10: Effect of silver nanoparticles (Ag-NPs) synthesized by neem leaf extract on the growth of *Sclerotium cepivorum* and *T. reesei in vitro*.

Table 3 data indicates that dipping onion transplants in all silver nanoparticles produced by *T. reesei* (Ag-NPs -Tr) decreased onion white rot disease infection. With regards to this, *T.*

reesei produced silver nanoparticles (Ag-NPs -Tr) at 250 µL/Land Flumid 24% resulted in the most decrease of onion white rot incidence (11.1%) and the highest yield of onion bulbs (256.3 and 258.8g/pot, respectively). Regarding plant height, the treatment involving *T. reesei* without silver nitrate and Flumid 24% fungicide had the biggest impact (63.7cm). Nevertheless, there was no discernible difference in plant height across all treatments.

Table 4 demonstrates that dipping onion transplants in all silver nanoparticles made from neem leaf extract (Ag-NPs -Ne) reduced infection with onion white rot disease as compared to the control. With regard to this, the application of silver nanoparticles produced from neem extract (Ag-NPs -Ne) at 250 µL/L and Flumid 24% resulted in the highest reduction in the occurrence of onion white rot (11.1%) and increased output of onion bulbs (258.4 and 258.8g/pot, respectively). When compared to the reference, the Flumid 24% treatment had the largest effect on plant height, followed by the Ag-NPs-Ne (Neem leaf extract) at 250 µL/L. However, there was no appreciable variation in plant height among the treatments. As a result, the current study demonstrated that Ag-NPs can concurrently reduce the percentage of onion white rot disease infection and enhance vegetative development and bulb output in greenhouse environments. Applying NPs to the roots of pre-infected onion plants successfully decreased white rot, enhancing onion plant development aspects and increasing host plant resilience.

Table 3: Effect of silver nanoparticles (Ag-NPs) synthesized by *T. reesei* on controlling white rot disease on onion under greenhouse conditions:

Treatment	Conc. µL/L	White rot incidence (%)	Plant height (cm)	Onion bulb yield g/pot
***T. reesie* AgNPs**	**125**	33.3	55.8	217.9
	250	11.1	59.6	256.3
	500	22.2	60.9	239.8
***T. reesie* without silver nitrate**	**125**	44.4	57.1	191.3
	250	33.3	58.4	199.0
	500	33.3	63.7	225.8
Celest FS 10%		22.2	61.2	235.5
Flumid24 %		11.1	63.7	258.8
Control (infected)		88.9	46.7	92.7
Control (healthy)		0.0	56.8	194.3
LSD at 0.05		**3.64**	**1.42**	**4.13**

Table 4: Effect of Silver Nanoparticles (Ag-NPs) synthesized by Neem extract on controlling white rot disease on onion under greenhouse conditions:

Treatment	Conc. µL/L	White rot incidence (%)	Plant height (cm)	Onion bulb yield g/pot
Neem AgNPs	**125**	22.2	56.3	204.1
	250	11.1	61.5	258.4
	500	33.3	58.1	251.8
Neem extract without silver nitrate	**125**	55.5	56.0	184.4
	250	44.4	57.2	202.1
	500	33.3	57.8	208.4
Celest FS 10%		22.2	61.2	235.5
Flumid 24 %		11.1	63.7	258.8
Control (infected)		88.9	43.2	92.7
Control (healthy)		0.0	56.8	194.3
LSD at 0.05		**3.99**	**1.15**	**3.21**

Discussion

The suppression of *S. cepivorum* by Ag-NPs from neem leaf extract can be attributed to its inhibitory effect on mycelial growth.

Consistent with findings reported by Jadhav *et al.,* (2016), which suggest that smaller nanoparticle size correlates with enhanced activity, it is plausible that the smaller size of the nanoparticles contributes to the heightened control of mycelial development observed with Ag-NPs derived from both Trichoderma filtrate and neem leaf extract. When compared to the reference, the Flumid 24% treatment had the largest effect on plant height, followed by the Ag-NPs-Ne (Neem leaf extract) at 250 µL/L. However, there was no appreciable variation in plant height among the treatments. As a result, the current study demonstrated that Ag-NPs can concurrently reduce the percentage of onion white rot disease infection and enhance vegetative development and bulb output in greenhouse environments. Applying NPs to the roots of pre-infected onion plants successfully decreased white rot, enhancing onion plant development aspects and increasing host plant resilience. Another important consideration is that the filtrate utilized in the nanoparticle synthesis process originated from a culture where hydrolytic enzymes were stimulated. This factor may contribute to the breakdown of the cell walls of the plant pathogen. Notably, an enzyme known as nitrate reductase appears to play a pivotal role in reducing Ag+ ions and generating metallic silver nanoparticles as a byproduct during the dissociation process from silver nitrate. Similar findings were reported in a study involving the fungus Fusarium oxysporum (Klittich and Leslie, 1988), where nitrate reductase was identified as responsible for both the formation of silver nanoparticles and the reduction of Ag+ ions. The inability of Ag-NPs to inactivate sulfhydryl groups in the fungal cell wall, thereby avoiding the production of insoluble substances, underscores their antifungal activity. This mechanism, coupled with the disruption of lipids and enzymes attached to membranes leading to cell lysis, has been reported by Duran *et al.,* (2005). Ag-NPs have been shown to exhibit antifungal activity by rupturing the membrane integrity of the pathogen, as demonstrated by Elgorban *et al.,* (2016). Moreover, previous research by Krishnaraj *et al.,* (2012) has indicated a higher antifungal effect of Ag-NPs against various phytopathogenic fungi. Additional studies by Duran *et al.,* (2016) and Ottoni *et al.,* (2017) suggest that the antimicrobial process may involve the creation of holes by attaching Ag-NPs to external proteins, thereby preventing DNA replication, or by releasing reactive oxygen species.

Conclusions

Based on the aforementioned findings, it can be inferred that the utilization of nanoparticles (NPs) synthesized from Trichoderma reesei filtrate (referred to as Ag-NPs-Tr) or neem leaf extract (referred to as Ag-NPs-Ne) on onion plant roots infected with white rot disease effectively reduced disease incidence. This reduction was achieved through the enhancement of host plant resistance and improvement of various developmental characteristics in onion plants. Consequently, this study holds the potential to pave the way for the adoption of biosynthesized NPs as an alternative to conventional fungicides, thus contributing to the reduction of environmental pollution. Moreover, such a shift could mitigate the health risks associated with the use of chemical fungicides.

Author Contributions:

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