

Biochemical Changes and Residues of Silver in Onion Plants Infected with *Sclerotium Cepivorum* and Treated with Green Silver Nanoparticles Synthesized by Neem Extract and *Trichoderma reesei* Compared with some Fungicides

Heba E. Aboelmagd¹, Hala A. Mahdy² and Naeema G. Hassan¹

ABSTRACT

Sclerotium cepivorum poses is danger disease to onion production, leading to considerable yield and financial losses. This study examined the effectiveness of bio-synthesized silver nanoparticles synthesized using *Trichoderma reesei* (*T. reesei* Ag-NPs) and Neem extract (Neem Ag-NPs). It also evaluated the associated biochemical changes, including enzyme activities, phenolic compounds, flavonoids, and protein content. The results showed that treatment with *T. reesei* Ag-NPs or Neem Ag-NPs reduced disease incidence and increased both fresh and dry weight, along with higher levels of phenolic compounds compared to the control. Moreover, the activities of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and chitinase were elevated in treated plants relative to the untreated control. The effect of (Ag-NPs) synthesized by Neem extract and *T. reesei* on PAGE of protein-infected onion plants with the white rot pathogen under greenhouse conditions revealed that onion bulbs contain 12 protein bands with molecular weights ranging from 122 to 11 kDa. The data indicates that all bands have appeared only in plants treated with *T. reesei* Ag-NPs. However, the band with 120 kDa was absent from all treatments except *T. reesei* Ag-NPs and control. In contrast, the 18 kDa band was absent from plants treated with Flumid 24% and control. The increased intensity of the induced protein was found in *T. reesei* Ag-NPs and Flumid 24% treated plants. Amounts of silver nanoparticles were identified with minimal residue in onion bulbs below permissible levels.

Key words: Silver nanoparticles, *Sclerotium cepivorum*, biochemical changes.

INTRODUCTION

Onions (*Allium cepa* L.) is essential for both local consumption and exports, with great economic significance on a global scale. However, in recent years there has been a major reduction in onion yield, mostly attributable to the destructive white rot disease produced by *S. cepivorum* (Elshahawy *et al.*, 2019). The disease shows observable symptoms above ground, including wilting, yellowing of older leaves, and die-back of leaf tips. Finally, collapse and decay occur as the symptoms are already along the leaf blades. Simultaneously, watery decay, White mycelia development at the bulb

base is indicative of a root infection below earth. These mycelia mats produce a large number of sclerotia, which are the primary source of inoculum for the upcoming crops (Darwesh and Elshahawy, 2021). This disease poses a serious risk to the production of onions, resulting in large yield losses and financial setbacks. As Ahmed and Ahmed (2015) point out; it has become quite common in Egypt, causing serious harm and occasionally a 100% crop loss. In particular, this disease threat poses a significant obstacle to the production of onions, especially in Upper Egypt (Mohamed, 2012). While standard fungicides are a reliable but not necessarily environmentally friendly treatment, researchers looking into approaches to manage white rot disease take into account both environmental and effective factors (Ahmed *et al.*, 2017). A promising approach, (Ag-NPs) has garnered a lot of interest due to their documented antifungal qualities and their uses against tomato early blight disease (Fares *et al.*, 2023).

Nanoparticles are capable of preventing disease development and transmission, such as *Sclerotium cepivorum* (Darwesh and Elshahawy, 2021). NPs can act as targeted carriers, delivering chemicals to particular plant cellular organelles, such as fertilizers, insecticides, herbicides, or genes (Nair *et al.*, 2010). Notwithstanding the possible advantages, little is known about NP toxicity in plants, investigations have looked into the mechanisms by which NPs affect plant growth. However, several studies have explored how nanoparticles influence plant growth through physiological, pharmacological, and molecular mechanisms (Siddiqui *et al.*, 2015). Additionally, it is crucial to comprehend their role and impact on oxidative enzymes and chemical properties in order to comprehend their efficacy. Accordingly, *Sclerotium cepivorum*-induced onion white rot was controlled by green produced silver nanoparticles (Mahdy and Abo-Elmagd, 2024). Additionally, their impact on oxidative enzyme activity is significant and useful in comprehending their function in reducing the occurrence of disease and promoting plant growth. Therefore, optimizing the effect of nanoparticles on host plants requires a thorough understanding of the

DOI: 10.21608/asejaiqsae.2025.434622

¹Plant Pathology Dept., Fac. Agric., Benha Univ., Egypt.

²Plant Pathology Res. Inst., Agric. Res. Center, Giza, Egypt.

Received, May 20, 2025, Accepted, June 22, 2025.

underlying mechanisms (Siddiqui *et al.*, 2015). *Azadirachta indica* leaf extract-derived biosynthesized AgNPs show great promise as a non-toxic and environmentally benign substitute for antimicrobial applications (Latif *et al.*, 2025). This study aims to investigate the biochemical changes in onion plants treated with green-synthesized silver nanoparticles in comparison to conventional fungicides.

MATERIAL AND METHODS

Source of *Trichoderma reesei*:

The tested *Trichoderma reesei* (AUMC5829) isolate used in this study was kindly obtained from Plant Pathology Dept., Fac. of Agric., Mansoura Univ. Egypt.

Source of the pathogen:

Virulent *Sclerotium cepivorum* isolate was obtained kindly from Plant Pathology Inst., Agric. Res. center, Giza, Egypt.

Nanoparticle Preparation and Characterization

Green-synthesized of silver nanoparticles were prepared using Neem leaf extract and the fungus *Trichoderma reesei* (AUMC 5829), and their characteristics were analyzed. The characterization techniques included UV-Visible spectroscopy, Dynamic Light Scattering (DLS), Zeta Potential analysis, and Transmission Electron Microscopy (TEM), as reported by Mahdy and Abo-Elmagd (2024).

Greenhouse Experiments:

After thoroughly mixing loamy sand soil (3 parts clay: 1-part sand, w/w) with a 5% commercial formalin solution, the soil was sterilized by covering it with polyethylene sheets for two weeks. Following sterilization, the polyethylene cover was removed, and the soil was aerated for ten days to allow complete evaporation of the formalin. Plastic pots (approximately 30 cm in diameter) were sterilized using the same method by immersing them in a 5% formalin solution. The pots were then filled with the pre-sterilized soil. An aggressive isolate of *Sclerotium cepivorum* was cultured on PDA plates at $20 \pm 2^\circ\text{C}$ for 10 days, and then transferred to sterile barley sand medium and incubated for two weeks. To ensure even distribution of the inoculum, the potted soil was thoroughly mixed with *S. cepivorum* at a concentration of 2.0% (w/w).

Greenhouse Evaluation of Neem and *T. reesei*-Based Silver Nanoparticles as Dipping Treatments against Onion White Rot:

To assess the effectiveness of silver nanoparticles synthesized using *Trichoderma reesei* (AUMC 5829) and Neem (*Azadirachta indica*) leaf extract in controlling onion white rot, a greenhouse experiment was conducted. Both extracts were used in nanoparticle form (Ag-NPs) and in their original form (without silver

nitrate) and compared with standard chemical fungicides.

Dipping treatments were prepared at three concentrations: 125, 250, and 500 $\mu\text{L/L}$ for each of the following: Neem extract with and without Ag-NPs, and *T. reesei* with and without Ag-NPs.

Two commercial fungicides were included as chemical controls:

- **Flumid 24%** (2',6'-Dibromo-2-methyl-4'-trifluoromethoxy-4-trifluoromethyl-1,3-thiazole-5-carboxanilide), applied at 80 mL per 100 kg of bulbs.
- **Celest FS 10%** (4-(2,2-Difluorobenzo [1,3] dioxol-4-yl)-1H-pyrrole-3-carbonitrile), applied at 1.5 cm^3 per kg.

Healthy onion seedlings (Giza 20 cultivar), 45 days old, were dipped in the respective treatment solutions for 2 hours prior to transplanting. Treated seedlings were then transplanted into 30 cm diameter plastic pots containing loamy sand soil previously infested with *Sclerotium cepivorum* inoculum at a concentration of 2% (w/w). Control plants were dipped in distilled water only. Each treatment was applied to three replicate pots, with three seedlings planted per pot. Disease assessment and plant response were monitored throughout the experiment under greenhouse conditions.

Disease assessment:

At 100 days after transplanting (DAT), disease incidence (DI %) was evaluated using the formula by Brix and Zinkernagel (1992):

$$\text{DI (\%)} = (\text{Number of infected plants} / \text{Total number of plants}) \times 100$$

Plant Growth and Yield Measurement

Plant height (cm) and fresh weight (g) were measured at 100 DAT. Onion bulbs were harvested from each pot and weighed to assess yield.

Biochemical Analysis

Enzyme Activity Assays

Bulb samples were collected for enzyme extraction following Tuzun *et al.* (1989). The following defense-related enzyme activities were determined:

Peroxidase (PO) absorbance was measured at 425 nm over 15 minutes (Allam and Hollis, 1972). Polyphenol Oxidase (PPO) assayed using the method of Matta and Dimond (1963). Phenylalanine Ammonia-Lyase (PAL) determined as per Dickerson *et al.* (1984). Chitinase activity measured following Ried and Ogryd-Ziak (1981).

Determination of phenolic compounds:

Total phenolics were quantified spectrophotometrically at 520 nm following the method of Bary and Thorpe (1954).

Determination of flavonoid content:

Flavonoids were determined spectrophotometrically at 420 nm following the method of Peixoto Sobrinho *et al.* (2008).

Protein Analysis and Residual Silver Determination

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Protein profiles of onion bulb samples were analyzed by SDS-PAGE following the protocol of Laemmli (1970). Briefly, total protein extracts were prepared and subjected to electrophoresis on 12% polyacrylamide resolving gels. Electrophoresis was performed under denaturing conditions to separate proteins based on their molecular weight. After electrophoresis, gels were stained with Coomassie Brilliant Blue, then imaged, scanned, and analyzed using the Gel Doc system (VILBER LOURMAT) to determine the presence and intensity of protein bands (Novello *et al.*, 2021).

Determination of residual silver ions in onion bulbs by inductively coupled plasma atomic emission spectrometry (ICP OES) analysis.

Onion bulbs treated with silver nanoparticles synthesized by two methods (biological and plant extract) in addition to untreated control were chosen to determine the residual metal ions. Samples were collected after 100 days of transplanting. The samples were meticulously wrapped in polyethylene bags and collected by hand while wearing vinyl gloves for protection (Allam *et al.*, 2003). For 24 hours, the samples are oven-dried at 80 degrees Celsius. Drying the gathered plant materials is recognized to be crucial because it prevents microbial degradation and guarantees a consistent reference value by calculating dry weight as opposed to fresh weight, which is challenging to measure (Markert, 1993 and Aksoy *et al.*, 2005). All of the materials were ground in a micro-hammer cutter and sieved through a 1.5-mm sieve to guarantee the even distribution of metals in the sample. Powdered materials that had been dried and ground were stored in sterile plastic vials (Demirezen and Aksoy 2006).

Analytical Techniques:

Microwave digestion

All samples were digested to provide an acceptable matrix for measuring metal ions, as well as an adequate and consistent recovery that was compatible with the analytical method (APHA, 2017). The Anton-Paar

microwave digestion system (Multiwave PRO) was used to digest the samples in an acid solution using 5 mL of 65% HNO₃ as an acid reagent. The Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES with Agilent Vapor Generation Accessory VGA 77 was used to determine the metal ions. For each series of measurements, an intensity calibration curve was constructed using a blank and three or more Merck Company (Germany) standards. The National Institute of Standards and Technology (NIST) provides standard reference materials and a quality control sample were utilized to verify the instrument reading, and external reference standards from Merck were used to verify the accuracy and precision of the metal ion readings.

Statistical Analysis:

Statistical analysis of all the previously designed experiments were carried out according to the procedures (ANOVA) reported by Snedecor and Cochran (1989) using Costat program (v. 6.3 Co Hort software, California, USA). Treatment means were compared by the least significant difference test (LSD) at a 5% probability level.

RESULTS AND DISCUSSION

Effect of *T. reesei* Ag-NPs on controlling white rot disease on onion under greenhouses conditions.

According to the data in Table 1, dipping onion transplants in all concentration of *T. reesei* Ag-NPs reduced infection with onion white rot disease. In this regard, *T. reesei* Ag-NPs at 250 µL/L and Flumid 24% gave the greatest reduction in onion white rot incidence (22.2%) and enhanced onion bulb production (252.3 and 254.5g/pot, respectively). In this regard, treatment with *T. reesei* without silver nitrate at 500 µL/L and Flumid 24% fungicide resulted in the greatest effect on plant height (62.7cm). However, no significant change in plant height was seen between all treatments. These results are in harmony with the results of Jung *et al.* (2010) which found that under greenhouse experiments Nano-silver liquids increased biomass and dry weights of green onion compared with control treatment. Elshahawy *et al.* (2018) found that by using Ag-NPs, tomato plants were shielded from the oomycete *Pythium aphanidermatum*, which causes sudden death disease. The reduced fresh and dried plant weights further indicate the pathogen's detrimental impact for the Giza 20 onion cultivar. Elshahawy *et al.* (2018) revealed similar findings, demonstrating a relationship between disease severity and declining plant growth in *S. cepivorum* infected onion crops.

Additionally, the findings demonstrated that both the Neem Ag-NPs and the *T. reesei* Ag-NPs at different concentrations improved plant growth and disease reduction. Furthermore, data show that the *S. cepivorum*

isolate is pathogenic and has a significant impact on the Giza 20 onion cultivar in greenhouse. On the other hand, using Flumid 24% fungicide gave the lowest percentage of incidence with onion white rot (22.2%) as the effect with Ag-NPs, (22.2%) and increased onion bulb yield.

Effect of Neem Ag-NPs on controlling white rot disease on onion under greenhouse conditions.

Data in Table 2 reveal that, dipping onion transplants in all concentrations of Neem Ag-NPs reduced infection with onion white rot disease compared to the control. In this regard, Neem Ag-NPs at 250 $\mu\text{L/L}$ and Flumid 24% yielded the greatest reduction in onion white rot incidence (22.2%) and enhanced onion bulb production (254.1 and 254.5g/pot, respectively). Nanomaterial's enhanced surface contact with cells causes reactions that produce (ROS), break down cell membranes, and damage proteins, giving them potent antibacterial properties. Antimicrobials based on nanomaterial's are resistant to pathogens because of -these interactions (Kalia *et al.*, 2020). In this regard, the treatment with Flumid 24% had the greatest effect on plant height. However, no significant change in plant height was seen between all treatments. As a

result, the current study demonstrated Ag-NPs' potential to reduce the proportion of infection with onion white rot disease while also improving vegetative development and bulb yield under greenhouse settings. Application of NPs to pre-infected onion plant roots successfully decreased white rot by raising host plant resistance and improving onion plant development characteristics. This might be as a result of silver nanoparticles' ability to stop white rot damage (Sharon *et al.*, 2010). In comparison to the untreated control, Salama (2012) discovered that the treatment of Ag-NPs at 60 mg/L increased the common bean's fresh and dry weights and corn seedlings. Nanotechnology offers special uses in biotechnology and agriculture, and the concentration of NPs is essential to their efficacy (Zhao *et al.*, 2020). According to Nair *et al.* (2010), NPs can act as useful transporters, delivering chemicals such as fertilizers, insecticides, herbicides, or genes to particular plant cellular organelles. Fungicides, such as those tested in this study, have been shown to interfere with crucial cellular processes in fungi, including cell wall synthesis and energy production (Shabana *et al.*, 2015 and Eliwa *et al.*, 2018).

Table 1. Effect of *T. reesei* Ag-NPs on controlling white rot disease on onion under greenhouses conditions

| Treatment | Conc. $\mu\text{L/L}$ | White rot incidence (%) | Plant height (cm) | Onion bulb yield g/pot |
|--------------------------------------------|--------------------------|----------------------------|-------------------|---------------------------|
| <i>T. reesei</i> Ag-NPs | 125 | 44.4 | 55.1 | 210.6 |
| | 250 | 22.2 | 59.0 | 252.3 |
| | 500 | 33.3 | 60.2 | 233.5 |
| <i>T. reesei</i> without silver nitrate | 125 | 55.6 | 56.4 | 187.9 |
| | 250 | 44.4 | 57.8 | 194.6 |
| | 500 | 44.4 | 62.7 | 220.8 |
| Celest FS 10% | | 44.4 | 60.4 | 232.2 |
| Flumid24 % | | 22.2 | 62.7 | 254.5 |
| Control (infected) | | 88.9 | 42.3 | 89.7 |
| Control (healthy) | | 0.0 | 56.1 | 190.9 |
| LSD at 0.05 | | 20.2 | 4.8 | 11.0 |

Table 2. Effect of Neem Ag-NPs on controlling white rot disease on onion under greenhouse conditions

| Treatment | Conc. $\mu\text{L/L}$ | White rot incidence (%) | Plant height (cm) | Onion bulb yield g/pot |
|----------------------------------------|--------------------------|----------------------------|----------------------|---------------------------|
| Neem Ag-NPs | 125 | 33.3 | 55.1 | 203.5 |
| | 250 | 22.2 | 61.2 | 254.1 |
| | 500 | 33.3 | 57.1 | 249.8 |
| Neem extract without silver nitrate | 125 | 55.6 | 54.8 | 181.1 |
| | 250 | 44.4 | 56.5 | 198.7 |
| | 500 | 33.3 | 56.1 | 205.3 |
| Celest FS 10% | | 44.4 | 62.7 | 232.2 |
| Flumid 24 % | | 22.2 | 60.4 | 254.5 |
| Control (infected) | | 88.9 | 42.3 | 89.7 |
| Control (healthy) | | 0.0 | 56.1 | 190.9 |
| LSD at 0.05 | | 12.0 | 3.5 | 12.2 |

Effect of *T. reesei* Ag-NPs on defense-related enzyme activities in infected onion plants with white rot pathogen under greenhouse conditions:

Data in Table 3 reveal that, in comparison to the control treatment, all tested treatments considerably raised the activity of the enzymes PO, PPO, PAL and chitinase. Flumid 24% was the most effective treatment, increasing the enzymes activity of PO, PPO, PAL, and chitinase enzymes by 419.6, 356.8, 435.9, and 438.0 % respectively, followed by *T. reesei* Ag-NPs 250 µL/L where it increased the activities of PO, PPO, PAL and chitinase enzymes by 404.1, 348.6, 425.6 and 330.4 % respectively, however, *T. reesei* Without silver nitrate the least effective dose was 125 µL/L. Many researchers have found the instability of both POD and PPO activities by using different dose of silver nanoparticles (Xihong *et al.*, 2011 and Raigond *et al.*, 2017). The pathogenic of *Alternaria solani* can be reduced by created Ag-NPs, according to Kumari *et al.* (2017). They also observed that pretreatment of particles on leaves boosted host resistance by boosting the antioxidant.

Effect of Neem Ag-NPs on defense-related enzyme activities in onion plants infected with white rot pathogen under greenhouse conditions:

Data in Table 4 reveal that, in comparison to the control treatment, all tested treatments considerably raised the activities of the enzymes PO, PPO, PAL and chitinase. The most effective treatment was Flumid 24%, which increased the activities of PO, PPO, PAL, and chitinase enzymes by 419.6, 356.8, 435.9, and 438.0 %, respectively. However, Neem without silver nitrate 125 µL/L was the least effective treatment. These

findings are consistent with those of Karim *et al.* (2012), who discovered that peroxidase enzyme activity was enhanced by low concentrations of novel NPs (gold and silver). Cell wall strengthening and the synthesis of specific bioactive substances, such as lignin, quinones, and melanin, which serve as a barrier against incoming pathogens by eliminating their pectolytic enzymes, may be linked to the elevated activity of PO, PPO, and PAL (Fugate *et al.*, 2016). Seed quality has been shown to improve with the use of copper (Cu) and zinc oxide (ZnO) nanoparticles (Khafaga *et al.*, 2019). Growth parameters, relative water content, and biochemical parameters such as glutathione S-transferase, (CAT), (POD), and total phenolic contents all increased with this treatment (Chung *et al.*, 2019). In a similar vein, applying 1.5 mg/L of ZnO NPs below 30 nm to chickpea plants considerably raised their overall biomass in comparison to 10 mg/L. Reduced relative water levels in plant leaves were associated with higher concentrations (mg/L) (Burman *et al.*, 2013).

Effect of *T. reesei* Ag-NPs on phenol content (mg/g fresh weight) in onion plants infected with white rot pathogen under greenhouse conditions:

The results in Table 5 show that, the application of Ag-NPs had a significant impact on the phenol content. All concentrations of *T. reesei* Ag-NPs increased the phenol compared with the control. Total and free phenol levels increased most significantly with Flumid 24% (316.0 and 260.2%), respectively. However, *T. reesei* without silver nitrate (125µL/L) was the least effective, increasing total and free phenols by 157.5 and 97.1%, respectively.

Table 3. Effect of *T. reesei* Ag-NPs on defense-related enzyme activity in onion plants infected with the white rot pathogen in greenhouse

| Treatment | Conc. µL/L | PO | PPO | PAL | Chitinase | Efficacy % | | | |
|-----------------------------------------|---------------|------|------|------|-----------|------------|-------|-------|-----------|
| | | | | | | PO | PPO | PAL | Chitinase |
| <i>T. reesei</i> Ag-NPs | 125 | 37.2 | 10.0 | 15.9 | 30.8 | 283.5 | 170.3 | 307.7 | 234.8 |
| | 250 | 48.9 | 16.6 | 20.5 | 39.6 | 404.1 | 348.6 | 425.6 | 330.4 |
| | 500 | 40.9 | 13.8 | 4.6 | 38.7 | 321.6 | 273.0 | 17.9 | 320.7 |
| <i>T. reesei</i> without silver nitrate | 125 | 23.5 | 7.3 | 13.7 | 20.0 | 142.3 | 97.3 | 251.3 | 117.4 |
| | 250 | 28.7 | 10.6 | 6.9 | 25.1 | 196.6 | 186.5 | 76.9 | 172.8 |
| | 500 | 29.0 | 11.3 | 15.5 | 28.4 | 199.0 | 205.4 | 297.4 | 208.7 |
| Celest FS 10% | | 18.1 | 4.8 | 11.8 | 30.2 | 86.6 | 29.7 | 202.6 | 228.3 |
| Flumid24 % | | 50.4 | 16.9 | 20.9 | 49.5 | 419.6 | 356.8 | 435.9 | 438.0 |
| Control (infected) | | 9.7 | 3.7 | 3.9 | 9.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| Control (healthy) | | 21.9 | 11.5 | 12.0 | 32.7 | 125.8 | 210.8 | 207.7 | 255.4 |

PO=Peroxidase, PPO= poly phenol oxidase, PAL= Phenylalanine ammonia-lyase

Table 4. Effect of Neem Ag-NPs on defense-related enzyme activities in onion plants infected with white rot pathogen under greenhouse conditions

| Treatment | Conc. μL/L | PO | PPO | PAL | Chitinase | Efficacy % | | | |
|-------------------------------------|---------------|------|------|------|-----------|------------|-------|-------|-----------|
| | | | | | | PO | PPO | PAL | Chitinase |
| Neem Ag-NPs | 125 | 29.3 | 14.1 | 13.7 | 34.2 | 202.1 | 281.1 | 251.3 | 271.7 |
| | 250 | 33.6 | 15.8 | 18.5 | 48.0 | 246.4 | 327.0 | 374.4 | 421.7 |
| | 500 | 29.0 | 12.7 | 14.9 | 44.4 | 199.0 | 243.2 | 282.1 | 382.6 |
| Neem extract without silver nitrate | 125 | 19.0 | 6.8 | 7.3 | 15.6 | 95.9 | 83.8 | 87.2 | 69.6 |
| | 250 | 22.1 | 8.0 | 8.0 | 23.0 | 127.8 | 116.2 | 105.1 | 150 |
| | 500 | 25.5 | 9.7 | 15.2 | 28.7 | 162.9 | 162.1 | 289.7 | 212.0 |
| Celest FS 10% | | 18.1 | 4.8 | 11.8 | 30.2 | 86.6 | 29.7 | 202.6 | 228.3 |
| Flumid24 % | | 50.4 | 16.9 | 20.9 | 49.5 | 419.6 | 356.8 | 435.9 | 438.0 |
| Control (infected) | | 9.7 | 3.7 | 3.9 | 9.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| Control (healthy) | | 21.9 | 11.5 | 12.0 | 32.7 | 125.8 | 210.8 | 207.7 | 255.4 |

PO=Peroxidase, PPO= poly phenol oxidase, PAL= Phenylalanine ammonia-lyase

Table 5. Effect of *T. reesei* Ag-NPs on phenol content (mg/g fresh weight) in onion plants infected with white rot pathogen under greenhouse conditions

| Treatment | Conc. μL/L | Free Phenol | Conjugated Phenol | Total Phenol | Efficacy % | | |
|-----------------------------------------|---------------|-------------|-------------------|--------------|-------------|-------------------|--------------|
| | | | | | Free Phenol | Conjugated Phenol | Total Phenol |
| <i>T. reesei</i> Ag-NPs | 125 | 29.6 | 40.6 | 70.2 | 187.4 | 420.5 | 287.8 |
| | 250 | 31.4 | 42.5 | 73.9 | 204.9 | 444.9 | 308.3 |
| | 500 | 25.3 | 31.6 | 56.8 | 145.6 | 305.1 | 213.8 |
| <i>T. reesei</i> without silver nitrate | 125 | 20.3 | 26.4 | 46.6 | 97.1 | 238.5 | 157.5 |
| | 250 | 20.7 | 29.7 | 50.4 | 101.0 | 280.8 | 178.5 |
| | 500 | 21.1 | 30.9 | 51.9 | 104.9 | 296.2 | 186.7 |
| Celest FS 10% | | 28.1 | 27.0 | 55.1 | 172.8 | 246.2 | 204.4 |
| Flumid24 % | | 37.1 | 38.1 | 75.3 | 260.2 | 388.5 | 316.0 |
| Control (infected) | | 10.3 | 7.8 | 18.1 | 0.0 | 0.0 | 0.0 |
| Control (healthy) | | 21.0 | 16.7 | 37.6 | 103.9 | 114.1 | 107.7 |

However, the conjugated phenols were affected differently by the treatments. In this respect, *T. reesei* without silver nitrate 125 μL/L decreased the conjugated phenols by 238.5 %. However, *T. reesei* Ag-NPs 250μL/L increased the conjugated phenols by 444.9%. These results are consistent with Ahmed's (2011) findings, which reveal that cucumber plants grown in soil inoculated with *Fusarium oxysporum* f. sp. *cucumerinum* had higher total phenol content when cucumber seeds were treated with *T. viride*. The phenolic compound biosynthesis pathway may have been triggered by an increase in phenolic acid production utilizing Ag⁺ and both elicited and non-elicited gene expressions were up-regulated (Ag-NPs and AgNO₃) (Xing *et al.*, 2015).

Effect of Neem Ag-NPs on phenol content (mg/g fresh weight) in onion plants infected with white rot pathogen under greenhouse conditions:

The results in Table 6 show that treatment with Ag-NPs had a substantial effect on phenol content compared to the control. All concentrations of Neem

Ag-NPs enhanced phenol content. Flumid 24% (316.0%) resulted in the largest rise in total phenols. However, Neem without silver nitrate 250 μL/L was the least effective, increasing total phenols by 99.4 percent. Regarding free phenol, all concentrations of Neem Ag-NPs increased free phenols. Flumid 24% had the highest increase in free phenols by (260.2%). Neem without silver nitrate 125 μL /L was the least effective one. However, Neem Ag-NPs 250μL/L increased the conjugated phenols by 393.6%. These results agree with those of Azad *et al.* (2019) who reported that increasing silver and other five nanoparticle materials concentrations increased PO, PPO activities, and total phenol content. This highlights how crucial it is to precisely calibrate fungicide dosages for the best disease control and the least amount of disturbance to plant physiology (Willyerd *et al.*, 2015). The phenolic compounds in the studied fungicides showed a notable dose-dependent increase, highlighting their capacity to trigger the manufacture of secondary metabolites implicated in plant, whereas the infected control showed noticeably decreased phenolic levels, demonstrating the

pathogen's harmful effects (Shalaby and Horwitz, 2015). The present knowledge of plant defensive responses, which holds that activating secondary metabolites, such as phenolics, is a typical tactic to fend off pathogenic attacks, is compatible with this steady rise in phenolic compounds (Lattanzio *et al.*, 2009).

Effect of *T. reesei* Ag-NPs on flavonoids in onion plants infected with white rot pathogen under greenhouse conditions:

Data in Table 7 show that, treatment with Ag-NPs had a significant effect on flavonoids when compared to the control. All concentrations of *T. reesei* Ag-NPs increased the flavonoids. The increase of flavonoids was highly recorded with Flumid 24% (610.9%). However, *T. reesei* without silver nitrate 125 µL/L was the least effective and increased the flavonoids by 127.3%. Adding *Pseudomonas fluorescens* 9 and 10 to bio-primed faba bean seed treatment boosted of flavonoids according to Alemu and Alemu (2013). Furthermore,

Kessler *et al.* (2003) noted those flavonoids' antioxidant activity results from their capacity to reduce free radicals or chelate them to lessen their formation.

Effect of Neem Ag-NPs on flavonoids in onion plants infected with white rot pathogen under greenhouse conditions:

The results in Table 8 show that, flavonoids were significantly affected by the Neem Ag-NPs treatment in comparison to the control. All concentrations of Neem Ag-NPs increased the flavonoids. The highest increase of flavonoids was recorded in the case of Flumid 24% (610.9%). However, Neem without silver nitrate 125 µL/L was the least effective and increased the flavonoids by 56.4%. These results are in line with those of El Refai *et al.* (2018), who discovered that the total polyphenols, phenol fractionation, and flavonoid content in treated plants increased when garlic extract was used for the manufacture of NPs.

Table 6. Effect of Neem Ag-NPs on phenol content (mg/g fresh weight) in onion plants infected with white rot pathogen under greenhouse conditions

| Treatment | Conc. µL/L | Free Phenol | Conjugated Phenol | Total Phenol | Efficacy % | | |
|-------------------------------------|------------|-------------|-------------------|--------------|-------------|-------------------|--------------|
| | | | | | Free Phenol | Conjugated Phenol | Total Phenol |
| Neem Ag-NPs | 125 | 29.2 | 28.8 | 58.0 | 183.5 | 269.2 | 220.4 |
| | 250 | 36.6 | 38.5 | 75.1 | 255.3 | 393.6 | 315.0 |
| | 500 | 31.7 | 23.0 | 54.7 | 207.8 | 194.9 | 202.2 |
| Neem extract without silver nitrate | 125 | 26.1 | 10.7 | 36.8 | 153.4 | 37.2 | 103.3 |
| | 250 | 26.6 | 9.5 | 36.1 | 158.2 | 21.8 | 99.4 |
| | 500 | 27.2 | 11.0 | 38.2 | 164.1 | 41.0 | 111.0 |
| Celest FS10% | | 28.1 | 27.0 | 55.1 | 172.8 | 246.2 | 204.4 |
| Flumid24 % | | 37.1 | 38.1 | 75.3 | 260.2 | 388.5 | 316.0 |
| Control infected | | 10.3 | 7.8 | 18.1 | 0.0 | 0.0 | 0.0 |
| Control healthy | | 21.0 | 16.7 | 37.6 | 103.9 | 114.1 | 107.7 |

Table 7. Effect of *T. reesei* Ag-NPs on flavonoids in onion plants infected with white rot pathogen under greenhouse conditions

| Treatment | Conc. µL/L | Flavonoids | Efficacy % |
|-----------------------------------------|------------|------------|------------|
| <i>T. reesei</i> Ag-NPs | 125 | 18.8 | 241.8 |
| | 250 | 36.8 | 569.1 |
| | 500 | 19.7 | 258.2 |
| <i>T. reesei</i> without silver nitrate | 125 | 12.5 | 127.3 |
| | 250 | 19.0 | 245.5 |
| | 500 | 27.9 | 407.3 |
| Celest FS 10% | | 26.4 | 380 |
| Flumid 24 % | | 39.1 | 610.9 |
| Control (infected) | | 5.5 | 0.0 |
| Control (healthy) | | 23.1 | 320 |

The formation of bonds with plant cell wall components (Mahadevan and Sridhar, 1986), These findings also concur with those of Abdel-Aziz *et al.* (2014), who created Ag-NPs using leaf extract from *Chenopodium murale* and found that Ag-NPs incorporating plant extract had higher levels of total phenolic compounds and total flavonoids than the plant extract alone. When compared to silver nitrate or *C. murale* leaf extract alone, Ag-NPs incorporating leaf extract shown greater antioxidant and antibacterial activities. It is possible to draw the conclusion that *C. murale* leaf extract is useful for producing possible antioxidant and antibacterial Ag-NPs for usage in commercial settings. According to Girilal *et al.* (2018), chemically produced silver nanoparticles raise the plant's secreted protein, carbohydrate, and phenolic component levels.

Effect of Ag-NPs Neem and *T. reesei* on PAGE of protein in infected onion plants with white rot pathogen under greenhouse conditions:

The SDS (PAGE of protein) results shown in Table 9 and Fig. (1) reveal that onion plants contain 12 protein bands with molecular weights ranging from 122 to 11 kDa.

The data indicates that all bands have appeared only in plants treated with *T. reesei* Ag-NPs. A band with 120 kDa was absent from all treatments except *T. reesei* Ag-NPs and control treatments. Moreover, the band of 18 kDa was absent from plants treated with Flumid 24% and control. The increased intensity of the induced protein was found in *T. reesei* Ag-NPs and Flumid 24% treated plants. It can be concluded that *T. reesei* Ag-NPs and Flumid 24% have the high enzyme activity as shown in Table (10) and Fig. (1). These findings can be explained by the findings of Štefanić *et al.* (2018), who discovered that tobacco seedlings' proteomic response to

exposure to Ag-NPs (100 µL) showed that the vast majority of proteins involved in primary metabolism were upregulated. A number of proteins that exhibit differential expression following Ag-NPs exposure fall into the following categories: protein synthesis, defense, energy, carbohydrate metabolism and amino acid metabolism. Induced expression of these proteins improves energy generation and tolerance mechanisms to deal with silver toxicity, according to Mirzajani *et al.* (2014). It is present in *Oryza sativa*, and silver treatment increased the production of proteins like L-ascorbate peroxidase, and superoxide dismutase that are involved in the detoxification of ROS. The release of Ag⁺ from Ag-NPs disrupts cell communication by binding to the second messenger calcium receptor, as demonstrated by the expression of calcium proteins such as calmodulins 1 and 3, translationally controlled protein, and NAC-transcription factor. According to Vannini *et al.* (2013), exposure to Ag-NPs in *Eruca sativa* changed the expression of certain proteins involved in protein folding, which in turn changed particular cellular functions. Proteins linked to energy generation and vacuolar transport was also found to express differently.

Residues of silver ions in onion plants infected with white rot pathogen and treated with biosynthesized silver nanoparticles (ICP-OES Analysis)

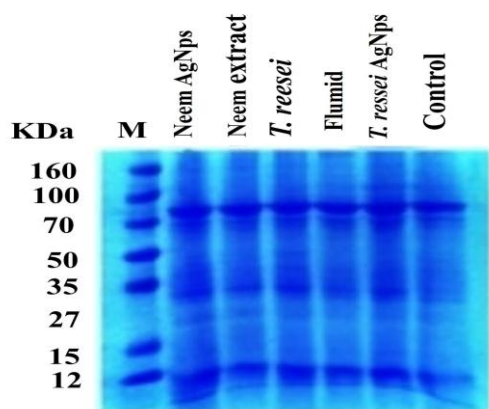
Data presented in Table 10 indicate the levels of silver ion residues in the tissues of onion bulbs treated with silver nanoparticles (Ag-NPs). The concentration of silver residues in onion bulbs increased proportionally with the applied concentration of Ag-NPs. Specifically, greenhouse treatments with *T. reesei* Ag-NPs and Neem Ag-NPs at 250 µL/L resulted in silver residues of 0.05 mg/kg, whereas treatments at 500 µL/L yielded residues of 0.1 mg/kg.

Table 8. Effect of Neem Ag-NPs on flavonoids (mg/g fresh weight) in onion plants infected with white rot pathogen under greenhouse conditions

| Treatment | Conc. µL/L | Flavonoids | Efficacy % |
|-------------------------------------|------------|------------|------------|
| Neem Ag-NPs | 125 | 15.1 | 174.5 |
| | 250 | 28.2 | 412.7 |
| | 500 | 9.4 | 70.9 |
| Neem extract without silver nitrate | 125 | 8.6 | 56.4 |
| | 250 | 9.0 | 63.6 |
| | 500 | 10.0 | 81.8 |
| Celest FS 10% | | 26.4 | 380 |
| Flumid 24 % | | 39.1 | 610.9 |
| Control (infected) | | 5.5 | 0.0 |
| Control (healthy) | | 23.1 | 320 |

Table 9. Effect of Ag-NPs Neem and *T. reesei* on PAGE of protein in infected onion plants with white rot pathogen under greenhouse conditions

| Band No | M.W Bp | Neem Ag-NPs | Neem extract | <i>T. reesei</i> | Flumid | <i>T. reesei</i> Ag-NPs | Control |
|---------|--------|-------------|--------------|------------------|--------|-------------------------|---------|
| | | 1 | 2 | 3 | 4 | 5 | |
| 1 | 120 | 0 | 0 | 0 | 0 | 1 | 1 |
| 2 | 86 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3 | 74 | 1 | 1 | 1 | 1 | 1 | 1 |
| 4 | 48 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5 | 38 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | 33 | 1 | 1 | 1 | 1 | 1 | 1 |
| 7 | 29 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | 18 | 1 | 1 | 1 | 0 | 1 | 0 |
| 9 | 16 | 1 | 1 | 1 | 1 | 1 | 1 |
| 10 | 14 | 1 | 1 | 1 | 1 | 1 | 1 |
| 11 | 12 | 1 | 1 | 1 | 1 | 1 | 1 |
| 12 | 11 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total | | 11 | 11 | 11 | 10 | 12 | 11 |



SDS-PAGE

Fig.1. Effect of Ag-NPs Neem and *T. reesei* on PAGE

These residue levels fall within safe and permissible limits for use. Mathematical modeling of silver nanoparticle bioaccumulation and bio distribution in the gastrointestinal tract, as described by Gmoshinski *et al.* (2013), employed first-order kinetic equations to characterize the internal exchange of these nanoparticles. Their findings indicated that oral administration of Ag-NPs at daily doses of 5–10 mg/kg body weight, either acutely or subacutely, can lead to potentially hazardous accumulation in vital organs such as the liver and spleen. The current results align with those reported by Abd-Elbaky *et al.* (2021), who demonstrated that silver nanoparticle residues in onion bulbs remained within acceptable safety margins and were minimal.

Table 10. Residual silver concentration in onion bulbs after treatment

| Treatment | Concentration (µL/L) | Residual Ag (mg/kg) |
|-------------------------|----------------------|---------------------|
| <i>T. reesei</i> Ag-NPs | 250 µL/L | 0.05 |
| <i>T. reesei</i> Ag-NPs | 500 µL /L | 0.1 |
| Neem Ag-NPs | 250 µL /L | 0.05 |
| Neem Ag-NPs | 500 µL /L | 0.1 |
| Control | - | 0.0 |

CONCLUSION

This study demonstrates the effectiveness of green-synthesized silver nanoparticles (Ag-NPs), derived from *Trichoderma reesei* and Neem extract, as environmentally friendly alternatives for controlling onion white rot caused by *Sclerotium cepivorum*. These nanoparticles not only suppressed disease incidence but also influenced key biochemical parameters such as total phenolic and flavonoid contents, along with oxidative enzyme activities, when compared to conventional fungicides. Traditional fungicides like Flumid 24% and Celest FS 10% exhibited strong inhibitory effects and remain effective chemical treatments. However, both *T. reesei* Ag-NPs and Neem Ag-NPs showed comparable disease control efficacy and significantly enhanced the biochemical defense mechanisms of onion plants.

The highest increases in total and free phenolic content were observed with Flumid 24% (315.1% and 261.0%, respectively), followed by *T. reesei* Ag-NPs at 250 µL/L (307.6% and 204.8%, respectively).

Overall, this research provides valuable insights into the biochemical responses of onion plants including changes in phenolic compounds, protein profiles, enzyme activity, and residual silver content following treatment with green-synthesized Ag-NPs. These findings pave the way for further exploration into the application of nanoparticles as sustainable tools in plant disease management.

REFERENCES

- Abdel-Aziz, M. S., M. S. Shaheen, A. A. El-Nekeety and M. A. Abdel-Wahhab. 2014. Antioxidant and antibacterial activity of silver nanoparticles bio created using *Chenopodium murale* leaf extract. J. of Saudi Chemical Society. 18(4): 356-363.
- Abd-Elbaky, A.A., S.E. EL-Nahas, M. E. Hassan and A. F. Desoukey. 2021. 'Effect of Silver Nanoparticles on Fusarium Basal Rot of Onion', International J. of Scientific and Engineering Research. 12 (4): pp. 481.
- Ahmed, G.A. 2011. Induction Resistance of Cucumber Plants (*Cucumis sativus* L.) Against Fusarium Wilt Disease Under Protected Houses Conditions. Almaty: Institute of Plant Protection and Quarantine, Kazakh National Agrarian University.
- Ahmed, H.A. and N.G. Ahmed. 2015. Management of white rot of onion using composts and *Trichoderma harzianum*. Curr. Life Sci. 1:63–69.
- Ahmed, M., M. Amin and I. El-Fiki. 2017. Efficacy of bioagents against *Alternaria porri* incitant of purple blotch of onion in Egypt. Egypt. J. Phytopathol. 45:17–29. doi: 10.21608/ejp.2017.89518.
- Aksoy, A. D. Demirezen and F. Duman. 2005. Bioaccumulation, detection and analyses of heavy metal pollution in Sultan Marsh and its environment. Water Air Soil Poll. 164: 241-255.
- Alemu, F. and T. Alemu. 2013. *Pseudomonas fluorescens* isolates as an inducer of physiological activities of faba bean (*Vicia faba*). African J. of Agricultural Research, 8(38):4864-487.
- Allam, A. I. and J. P. Hollis. 1972. Sulfide inhibition of oxidase in rice roots. Phytopathology. 62: 634-639.
- Allam, M.G.M., E.T. Snow and A. Tanaka. 2003. Arsenic and heavy metal contamination of vegetables grown in Samta Village, Bangladesh. Sci. Total Environ. 308:83-96.
- APHA: American Public Health Association. 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed. (Rice, E. W., Baird, R. B., Eaton, A. D., Clesceri, L. S. eds.) Washington DC.
- Azad, C.S., A.Kumar; G. Chand and R.D. Ranjan. 2019. Evaluation of host defense-inducing nanoparticles against *Alternaria tenuissima* (Kunze ex pers.) Wiltshire causing die-back disease of chili. J. Pharmacol. and Phytoch. 8(3): 222-226.
- Bary, H.G. and W.V. Thorpe. 1954. Analysis of phenolic compounds of interest in metabolism/Methods of chemical analysis. 1: 27-51.
- Brix, H. D. and V. Zinkernagel. 1992. Effects of cultivation, conditioning, and isolate on sclerotium germination in *Sclerotium cepivorum*. Plant Pathology. 41: 13–19. <https://doi.org/10.1111/j.1365-3059.1992.tb02309.x>
- Burman, U., M. Saini, P. Kumar. 2013. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicological and Environmental Chemistry. 95(4):605-612.
- Chung, I.M., K.Rekha, B.Venkidasamy and M. Thiruvengadam. 2019. Effect of copper oxide nanoparticles on the physiology, bioactive molecules, and transcriptional changes in *Brassica rapa* ssp. *rapa* seedlings. Water Air Soil Pollut. 230: 1–14.
- Darwesh, O.M. and I.E. Elshahawy. 2021. Silver nanoparticles inactivate sclerotial formation in controlling white rot disease in onion and garlic caused by the soil borne fungus *Stromatinia cepivora*. European J. of Plant Pathology. 160(4):917-934.
- Demirezen, D. and A.Aksoy. 2006. Heavy metal levels in vegetables in Turkey is within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb. J Food Quality. 29: 252-265.
- Dickerson, D.P., S.F.Pascholati, A.E.Hagerman, L.G. Butler and R.L. Nicholson. 1984. Phenylalanin ammonia-lyase and hydroxy cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium carbonum*. Physiol Plant Pathol. 25:111–123.
- Eliwa, M., M.E.S.Aly, H.Abd-Alla, A. Galal. 2018. Efficacy of certain fungicide alternatives for controlling sugar beet powdery mildew. J. Phytopath. Pest Manag. 5: 76–87.
- El-Refai, A. A., G. A.Ghoniem, A. Y. El-Khateeb and M. M. Hassaan. 2018. Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents. J. of Nanostructure in Chemistry. 8(1): 71-81.
- Elshahawy, I. E., N.Saied, F. Abd-El-Kareem and A. A. Morsy. 2018. Field application of selected bacterial strains and their combinations for controlling onion and garlic white rot disease caused by *Stromatinia cepivora*. Journal of Plant Pathology, 100: 493–503. <https://doi.org/10.1007/s42161-018-0113-z>.
- Elshahawy, I.E., A.A.Morsy, F.Abd-El-Kareem and N.M. Saied. 2019. Reduction of *Stromatinia cepivora* inocula and control of white rot disease in onion and garlic crops by repeated soil applications with sclerotial germination stimulants. Heliyon. 5: e01168.
- Fares, A., A. M. M.Mahdy, G. M. D.EL Habbaa, A. A. Abdalla and G. A. Ahmed. 2023. Biological Synthesis of Silver Nanoparticles Exposed to Gamma Irradiation for Control of Early Blight Disease in Tomatoes. Journal of Plant Protection and Pathology. 14(5):125-132
- Fugate, K. K., W. S.Ribeiro, E. C.Lulai, E. L. Deckard and F. L. Finger. 2016. Cold temperature delays wound healing in postharvest sugar beet roots. Front. Plant Sci. 7:499. doi: 10.3389/fpls.00499

- Girilal, M., A.M.Fayaz, L.K.Elumalai, A.Sathiyaseelan, J.Gandhiappan and P.T. Kalaichelvan. 2018. Comparative stress physiology analysis of biologically and chemically created silver nanoparticles on *Solanum lycopersicum* L. Colloid and Interface Sci. Communications. 24:1-6.
- Gmoshinski, I.V., S.A.Khotimchenko, V.O. Popov, B.B. Dzantiev, A.V. Zherdev, V.F. Demin and Y.P. Buzulukov. 2013. Nanomaterials and nanotechnologies: methods of analysis and control. Russian Chemical Reviews. 82(1): 48-76.
- Jung, J. H., S. W.Kim, J. S. Min, Y. J. Kim, K. Lamsal, K. S. Kim and Y. S. Lee. 2010. The effect of nano-silver liquid against the white-rot of green onion caused by *Sclerotium cepivorum*. Mycobiology. 38: 39–45.
- Kalia, A., K.A. Abd-Elsalam and K. Kuca. 2020. Zinc-based nanomaterial for diagnosis and management of plant diseases: ecological safety and future prospects. J. Fungi, 6:222. doi: 10.3390/jof6040222.
- Karim, Z., R.Adnan and M. S. Ansari. 2012. A low concentration of silver nanoparticles not only enhances the activity of horseradish peroxidase but alters the structure also. PloS one. 7(7): e41422.
- Kessler, M., G. Ubeaud and L. Jung. 2003. Anti-and pro-oxidant activity of rutin and quercetin derivatives. Journal of pharmacy and pharmacology. 55(1):131-142.
- Khafaga, A.F., M.E.Abd El-Hack, A.E.Taha, S.S.Elnesr and M. Alagawany. 2019. The potential modulatory role of herbal additives against Cd toxicity in human, animal, and poultry: A review. Environ. Sci. Pollut. Res. 26:4588–4604.
- Kumari, M., S.Pandey, A.Bhattacharya, A.Mishra and C. S. Nautiyal. 2017. Protective role of biocreated silver nanoparticles against early blight disease in *Solanum lycopersicum*. Plant Physiol. Biochem. 121:216–225. doi: 10.1016/j.plaphy.11.004.
- Laemmli, U.K. 1970. "Cleavage of structural proteins during the assembly of the head of bacteriophage T4." Nature (Lond). 227- 680.
- Latif, R., M. Y.Shani, A.Shazadi and M. Y. Ashraf. 2025. Antibacterial and antifungal activities of silver nanoparticles synthesized using neem (*Azadirachta indica*) leaf extract. Trends in Pharmacy. 2:1-8.
- Lattanzio, V., P.A.Kroon, S.Quideau, D.Treutter. 2009. Plant phenolics-Secondary metabolites with diverse functions. Recent advances in polyphenol research. 1:1–35.
- Mahadevan, A. and K.Sridhar. 1986. Methods of Physiological Plant 3 Rd Edition, Sivakami Pub. Madras.
- Mahdy, H. A. and H. E. Abo-Elmagd. 2024. Management of onion white rot caused by *Sclerotium cepivorum* Berk using bio-created silver nanoparticles. Egyptian J. of Crop Protection. 19(1):80-96.
- Markert, B. 1993. Plant as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment, Ed. B., Markert, VCH Weinheim, New York/Basel/Cambridge.
- Matta, A. and A.E. Dimond. 1963. Symptoms of Fusarium wilt in relation to the quantity of Fungus and enzyme activity in tomato stems. Phytopathology. 53:574 -587.
- Mirzajani, F., H. Askari, S. Hamzelou, Y. Schober, A. Römp, A. Ghassempour and B. Spengler. 2014. Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L. Ecotoxicol. Environ. Saf. 108:335-339.
- Mohamed, H.A. 2012. Integrated control of onion white rot disease. Master's Thesis, Benha University, Banha, Egypt, 134.
- Nair, R., S.H.Varghese, B.G.Nair, T.Maekawa, Y. Yoshida and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. Plant Sci. 179: 154–163.
- Novello, M.S., R.B. Silva and L.P. Souza. 2021. Quantitative profiling of onion bulb proteins under biotic stress by SDS-PAGE. J. Plant Physiol. 258:153367.
- Pexioto Sobrinho, T.J.S., K.C.M.Cardoso, T.L.B.Gomes, U.P.Albuquerque and E.L.C.Amorium. 2008. Validação de metodologia espectrofotométrica para quantificação dos flavonoides de Bauhinia cheilantha (Bongard) Steudel. Brazilian J. of Pharmaceutical Sci. 44(4):683-689.
- Raigond, P., B.Raigond, B.Kaundal. B.Singh, A.Joshi and S. Dutt. 2017. Effect of zinc nanoparticles on ant oxidative system of potato plants. J. of Environmental Biology. 38(3):435- 439.
- Reid, J. D. and D. M.Ogryd-Ziak. 1981. Chitinase overproducing mutant of *Serratia marcescens*. Appl. and Environ. Microbiol. 41(3): 664-669.
- Salama, H. M. H. 2012. Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). International Research J. of Biotechnology. 3:190–197.
- Shabana, Y., M.El-Boray, M.Mustafa and G.Al-Juboori. 2015. Antifungal activity of plant extracts, essential oils, and microbial culture filtrates against *Botrytis cinerea* in-vitro. J. Plant Prot. Pathol. 6:1297–1311.
- Shalaby, S. and B.A. Horwitz. 2015. Plant phenolic compounds and oxidative stress: Integrated signals in fungal–plant interactions. Curr. Genet. 61:347–357.
- Sharon, M., A. K. Choudhary and R. Kumar. 2010. Nanotechnology in agricultural diseases and food safety. J. of Phytotherapy. 2(4):83–92.
- Siddiqui, M.H., M.H.Al-Whaibi, M.Firoz and M.Y. Al-Khaishany. 2015. Nanotechnology and Plant Sciences. Springer International Publishing, Berlin/Heidelberg, Germany. Role of Nanoparticles in Plants. 19–35.
- Snedecor, G.W. and W.G. Cochran. 1989. Statistical methods. Oxford and J. PH. Publishing Com. 8th edition.
- Štefanić, P.P., P. Cvjetko, R.Biba, A.M. Domijan, I.Letofsky-Papst, M.Tkalec and B. Balen. 2018. Physiological, ultrastructural and proteomic responses of tobacco seedlings exposed to silver nanoparticles and silver nitrate. Chemosphere. 209:640-653.

- Tuzun, S., M. N.Rao, U.Vogeli, C. L.Schardl and J.Kuc. 1989. Induced systemic resistance to blue mold: early induction and accumulation of β -1, 3- glucanases, chitinases and other pathogenesis-related proteins (b-proteins) in immunized tobacco. *Phytopathology*. 79(9): 979-983.
- Vannini, C., G.Domingo, E.Onelli, B.Prinsi, M.Marsoni, L.Espen and M.Bracale. 2013. Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. *PloS one*. 8(7), e68752.
- Willyerd, K.T., C.A.Bradley, V.Chapara, S.Conley, P.Esker, L.Madden, K.Wise, P.Paul. 2015. Revisiting fungicide-based management guidelines for leaf blotch diseases in soft red winter wheat. *Plant Dis*. 99:1434–1444.
- Xihong, L.i., L.I.Weili, Y.Jiang, Y.Ding, J.Yun, Y.Tang and P. Zhang. 2011. Effect of nano-ZnO-coated active packaging on quality of fresh-cut „Fuji” apple. *Int. J. of Food Sci. and Technol*. 46:1947–1955.
- Xing, B., D.Yang, W.Guo, Z.Liang, X.Yan, Y. Zhu and Y. Liu. 2015. Ag+ as a more effective elicitor for production of tanshinones than phenolic acids in *Salvia miltiorrhiza* hairy roots. *Molecules*. 20(1):309–324.
- Zhao, L., Lu, L., A.Wang, H.Zhang, M.Huang, H.Wu, B.Xing, Z.Wang and R.Ji. 2020. Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. *J. of agricultural and food chemistry*. 68(7):1935-1947.

الملخص العربي

التغيرات الكيميائية الحيوية وبقايا الفضة في نباتات البصل المصابة بالفطر *Sclerotium cepivorum* والمعاملة بجسيمات الفضة النانوية (Ag-NPs) المخلقة خضريا بمستخلص النيم و *Trichoderma reesei* مقارنة ببعض المبيدات الفطرية

هبة عليوة أبو المجد، هالة عبده مهدي، نعيمة جمعة حسن

البروتين PAGE لنباتات البصل المصابة والمعاملة وقد أظهرت النتائج أن ١٢ نطاقا بروتينا بأوزان جزيئية تتراوح من ١٢٢ إلى ١١ كيلو دالتون موجودة في نباتات البصل. تشير البيانات إلى أن جميع النطاقات ظهرت فقط في النباتات المعالجة بـ *T. reesei* Ag-NPs. ومع ذلك، كان الشريط الذي يحتوي على ١٢٠ كيلو دالتون غائبا عن جميع المعاملات باستثناء *T. reesei* Ag-NPs ومعاملة المقارنة. علاوة على ذلك، كان الشريط الذي يحتوي على ١٨ كيلو دالتون غائبا عن النباتات المعاملة بـ Flumid ٢٤٪ كما تم العثور على زيادة كثافة البروتين المستحث في حالة المعاملة بـ *T. reesei* Ag-NPs و Flumid ٢٤٪. كما أشارت النتائج إلى أنه تم اكتشاف تركيزات من جزيئات الفضة النانوية مع وجود بقايا منخفضة في أبصال البصل المعاملة ضمن الحدود المقبولة.

الكلمات المفتاحية: جسيمات نانوية فضية، *Sclerotium cepivorum*، التغيرات الكيميائية الحيوية.

يؤثر الفطر الممرض *Sclerotium cepivorum* بشدة على زراعة البصل، مما يؤدي إلى خسائر كبيرة في المحصول. بحثت هذه الدراسة دراسة تصنيع جزيئات الفضة النانوية المركبة بيولوجيا باستخدام الفطر *Trichoderma reesei* (Tr-Ag-NPs) ومستخلص النيم (Ne-Ag-NPs) واختبار فعاليتها في مكافحة المرض والتغيرات الكيميائية الحيوية مثل المركبات الفينولية والفلافونويد ومحتواها من البروتين. حيث تم دراسة نشاط الإنزيمات المؤكسدة في وقت واحد. وقد أظهرت النتائج أن استخدام Tr-Ag-NPs أو Ne-Ag-NPs يقلل من حدوث المرض ويؤدي إلى زيادة الوزن الطازج والوزن الجاف بالإضافة إلى زيادة المركبات الفينولية مقارنة بتلك غير المعاملة، كما زادت أنشطة إنزيمات البيروكسيداز (PO) والبوليفينول أوكسيداز (PPO) وفينيل ألانين أمونيا لياز (PAL)، وإنزيمات الكيتيناز مقارنة بغير المعاملة. تمت دراسة تأثير جسيمات الفضة النانوية (Ag-NPs) التي تم تصنيعها بواسطة مستخلص النيم و *T. reesei* على محتوى