RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (3) 1275-1282, 2025
http://doi.org/10.54910/sabrao2025.57.3.38
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

BIOSYNTHESIZED SILVER NANOPARTICLES IMPACT ON THE FUNGI THAT CAUSE SPOT AND BLIGHT DISEASES IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

N.H. MOHAMMED[1*], M.A. FAYYADH[1], and S.SH. AL-LUAIBI[2]

[1]Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq
[2]Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq
*Corresponding author's email: najlaaallatifi@gmail.com
Email addresses of co-authors: mohammed.fayad@uobasrah.edu.iq, salah.al.labeia@hotmail.com

SUMMARY

The study aimed to assess the effectiveness of silver nanoparticles, synthesized from strawberry fruit extract, in inhibiting the growth of fungi responsible for spot and blight diseases in tomato (*Solanum lycopersicum* L.). The research identified 18 species of fungi linked to these symptoms on tomato foliage, notably *Alternaria alternata, Alternaria solani, Botrytis cinerea, Cladosporium cladosporioides, and Scytalidium lignospora,* using molecular ITS1-ITS4 gene analysis. The characterization of silver nanoparticles, with a size of 61.87 nanometers, proceeded through ultraviolet-visible and zeta potential measurements. Findings indicated these biosynthesized silver nanoparticles hindered the growth of all tested fungi. Notably, they exhibited greater efficacy against *Cladosporium,* with its growth inhibited by 86.4%. Inhibition percentages for other pathogenic fungi ranged between 75.06% and 81.11%.

Keywords: Tomato (*S. lycopersicum* L.), nanotechnology, plant diseases, silver nanoparticles

Key findings: The study found biosynthesized silver nanoparticles effectively inhibited the growth of various fungal pathogens responsible for spot and blight diseases in tomato (*S. lycopersicum* L.). Their notable efficacy against *Cladosporium* reached an inhibition rate of 86.4% and demonstrated a correlation between nanoparticle concentration and effectiveness.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: December 20, 2023; Accepted: February 05, 2025.

Citation: Mohammed NH, Fayyadh MA, Al-Luaibi SSH (2025). Biosynthesized silver nanoparticles impact on the fungi that cause spot and blight diseases in tomato (*Solanum lycopersicum* L.). *SABRAO J. Breed. Genet.* 57(3): 1275-1282. http://doi.org/10.54910/sabrao2025.57.3.38.

INTRODUCTION

The Basra Governorate stands as the primary hub for winter tomato (*Solanum lycopersicum* L.) production, boasting a cultivated area of four hectares in 2020, yielding approximately 206,556 tons of the said crop within the same year (Planning and Follow-up Department 2020-Basra Agriculture Directorate). However, the tomato plant faces a gamut of threats from fungal, bacterial, and viral infections. Among these, prevalent fungal diseases include early blight (from *Alternaria solani), Fusarium* vascular wilt (from *Fusarium oxysporum),* leaf spot (from *Alternaria alternata* and *Stemphylium* sp.), damping off, stem and fruit rot (caused by *Rhizoctonia solani*), and late blight (stemming from *Phytophthora infestans*) (Kirankumar *et al*., 2008; Fayyad and Abbas, 2018).

The impact of early blight disease, caused by *A. solani*, resonates profoundly across the Solanaceae family, resulting in substantial crop loss worldwide (van der Walls *et al*., 2001). Its symptoms manifest as concentrated, irregular spots on leaves, branches, and fruits, encircled by a yellow halo. The infection progresses to encompass entire leaf and branch areas, leading to their demise and subsequent fall. Necrotic spots emerge on stems and fruits, inducing tissue rot. Factors like high humidity, attributable to rain, dew, and excessive irrigation, notably contribute to disease development, particularly in agriculture systems sheltered by plastic covers (Trigiano *et al*., 2004). The resultant losses due to this disease fluctuate between 5%–50% (Chaudhary *et al*., 2021).

With a growing awareness of the adverse effects of chemical pesticides on the environment and human health, the quest for alternative methodologies has become a paramount focus of global scientific research. Nanotechnology emerges as a promising avenue in disease control owing to its minimal environmental impact, reduced human health risks, lower usage quantities per unit area, and potential application as carrier materials for active ingredients, alongside its potential in early disease pathogen detection (Gupta and Xie, 2018; Shang *et al*., 2019).

Nanotechnology, a facet of modern technology permeating various sectors, including agriculture, industry, and medicine, involves the synthesis of nanoparticles through diverse methods. Among these, biological synthesis, accomplished using microorganisms or plant extracts, stands out as an easy, swift, cost-effective, and eco-friendly approach. Researchers have utilized extracts from various plant parts (stems, leaves, fruits, root peels, and seeds) to produce nanoparticles with key minerals including silver, gold, zinc oxide, and copper oxide (Hatem *et al*., 2021).

The terms biosynthesis and green synthesis denote the methods employing single-celled microorganisms like bacteria and actinomycetes or multicellular microorganisms, such as yeasts, fungi, algae, and plant extracts, in nanoparticle synthesis. Biological methodologies offer inherent advantages—being more beneficial, safer, and cost-effective—by reducing pollution, energy consumption, and enhancing environmental safety and human health (Ying *et al*., 2022).

Silver nanoparticles, renowned for their antifungal and antibacterial properties and ability to stimulate systemic resistance in plants, hold a prominent position in controlling plant diseases (Holden *et al*., 2016). Biosynthesis of nanoparticles using plant extracts represents a prevalent method. For instance, Barman *et al*. (2020) utilized the aqueous extract of ginger (*Zingiber officinale* L.) roots for silver nanoparticle synthesis. Additionally, Ansiri *et al*. (2023) observed that silver nanoparticles synthesized using neem leaf extract significantly reduced early blight disease severity in tomato plants caused by *A. solani*, showing a 73% decrease in infection. Moreover, treated plants exhibited increased wet and dry weights alongside heightened antioxidant enzyme activity.

The tomato plant is significant as both a food source and an economic crop. Likewise, it is imperative to explore alternatives to chemical pesticides. Hence, this study endeavors to evaluate the efficacy of silver nanoparticle synthesis using a strawberry fruit extract in inhibiting the growth of fungi responsible for spot and blight diseases in tomatoes, as well as an alternative pesticide.

MATERIALS AND METHODS

Fungi extraction from infected tomato leaves

Fungi isolation transpired from tomato (*Solanum lycopersicum* L.) leaves affected by spot and blight infections. Separating the infected tomato parts, they incurred cutting into small 0.5-cm pieces after being washed with tap water. These segments underwent sterilization using a 10% sodium hypochlorite solution for two minutes, followed by rinsing with sterile distilled water. After drying with sterile filter paper, transferring every set of five pieces onto Petri dishes containing autoclaved PDA and V-8 media supplemented with 250 mg/liter of the antibiotic chloramphenicol used sterile forceps. Then, the dishes placed in an incubator at 25 °C ± 1 °C remained for 5–7 days. Subsequently, the fungal growth scrutiny used a compound microscope. The isolated fungi sustained purification on PDA and V-8 Agar for phenotypic identification at the genus and species levels, utilizing approved taxonomic keys (Ellis, 1971; Domsch *et al.*, 1976; Pitt and Hocking, 2009; Beer *et al.*, 2006; Crous *et al*., 2006; Phillips *et al*., 2013; Woudenberg *et al*., 2013).

Molecular diagnosis of fungi

The mycelium was four days old when harvested from the culture medium, then, crushed with liquid nitrogen in a sterile ceramic mortar, and subsequently used for DNA extraction. Utilizing the Geneaid-Plant Genomic DNA Mini Kit (GP100) following the manufacturer's protocol, DNA extraction ensued from 0.2 grams of mycelium powder. The concentration and purity of the DNA bore evaluation in nanograms per microliter using the NanoDrop device by Thermo Scientific. The samples' preservation continued at -20 °C for future analyses. For the ITS gene region analysis, the genomic DNA underwent amplification using ITS1-ITS4 primers. Sending the resulting gene amplification products to Macrogen for nucleotide sequence determination and subsequent comparison with sequences in the NCBI GenBank database.

Preparation of silver nanoparticles using strawberry fruit extract

Silver nanoparticles synthesis used a green method based on the process described by Umoren *et al*. (2017). Initially, 25 grams of fresh strawberries underwent washing with distilled water, followed by slicing. These reached combining with 100 ml of non-ionic distilled water before blending using an electric mixer. The resulting extract's filtration utilized Whatman No. 1 filter paper to eliminate impurities before centrifugation at 8000 rpm for 10 minutes. The clarified extract's storage in glass bottles had a temperature of 4 °C until further use in subsequent experiments.

Subsequently, preparing a 0.01 M solution of silver nitrate (AgNO3) followed. The addition of five milliliters of the aqueous strawberry fruit extract to a 250-ml flask containing 95 ml of the silver nitrate solution continued. This reaction mixture remained at room temperature, shielded from light, for a duration of 18 days. Over this period, the color change in the reaction mixture indicated the gradual formation of silver nanoparticles. Identification of these nanoparticles proceeded through measurements using a spectrophotometer and assessment of their Zeta potential.

Effect of biosynthesized nanosilver on fungi growth

Preparing a standard solution of biosynthesized nanosilver with a concentration of 1000 ppm materialized. The introduction of specific volumes of this standard solution occurred into a 1-liter flask containing 500 ml of PDA medium to achieve varied concentrations (10, 30, 50, 70, and 90). The determination of quantities of the standard solution added used the formula:

$$C1V1 = C2V2$$

Where C1 = initial concentration, V1 = initial volume, C2 = final concentration, and V2 = final volume.

After adding the calculated amounts of agar, the medium reached pouring into Petri dishes measuring 9 cm in diameter. Each concentration had three replicates for every fungus, accompanied by three additional dishes for the control treatment. Upon solidification of the medium, a 0.5 cm diameter disk from the edge of a fresh culture of each pathogenic fungus used in the experiment attained inoculation at the center of each dish. The dishes then received incubation at a temperature of 25 °C ± 1 °C until the growth of pathogenic fungi in the control treatment reached completion. Subsequently, taking the average measurement of two perpendicular growth diameters progressed, with the percentage of inhibition calculated (Shaaban and Al-Mallah, 1993).

Inhibition (%) = (growth rate in control - growth rate in treatment)/growth rate in control × 100

Statistical analysis

The laboratory experiments followed a completely randomized design, with all means compared using the least significant difference (LSD) at a probability level of 0.01, utilizing the statistical program Genstat (Al-Rawi and Khalaf-Allah, 1980).

RESULTS AND DISCUSSION

Fungi associated with pathological symptoms on the foliage of tomato (*Solanum lycopersicum* L.) plants succeeded investigation. The isolation of 18 species was evident, with notable presence from fungi, such *as A. alternata, A. solani, B. cinerea, C. cladosporioides,* and *S. lignospora*, marking their significance.

Molecular diagnosis, relying on the amplification of the ITS1-ITS4 gene region, identified these fungi as belonging to *A. solani*, *B. cinerea*, and *C. cladosporioides*. The comparison with reference isolates in GenBank revealed a strikingly high percentage of identity, reaching 99.82%, 100.00%, and 100.00% respectively. The nucleotide sequences have been duly recorded, and the isolates submitted to GenBank are available in Table 1.

Green synthesis of silver nanoparticles using strawberries

The electromagnetic spectrum region indicates the formation of silver nanoparticles, leading to a shift in the solution's color to dark brown, signifying nanosilver formation and enabling the measurement of zeta potential (Figure 1). Silver nanoparticles derived from strawberry fruit extract measured 61.87 nanometers. Biosynthesizing silver nanoparticles stands as a desirable pursuit, supported by Bhattacharjee *et al*. (2018), who explored nanosilver, chitosan, and silicon for *A. solani* and *A. alternata*. Ahmad (2017) also observed the inhibitory effects of silver nanoparticles on *A. alternata* and *B. cinerea*. Mostafa *et al*. (2021) discovered that silver nanoparticles derived from pomegranate and orange peel extracts effectively suppressed *A. solani*, responsible for tomato blight (Figure 2). Various explanations exist for nanosilver's antifungal efficacy, including hindering fungal cell membrane biosynthesis (Akpinar *et al.*, 2021), causing defects in the plasma membrane, disrupting the membrane's fatty nature and selective permeability, or impeding crucial enzyme functions within the cell. Additionally, nanosilver became implicated in inducing systemic resistance in plants (Rai *et al*., 2018).

Table 1. Species and strains and their accession numbers in GenBank isolated from tomato leaves.

Fungus	Stain	Accession number
Alternaria solani	A.s	OR887720
Botrytis cinerea	NH2	OR887732
Cladosporium cladosporioides	NH3	OR887733

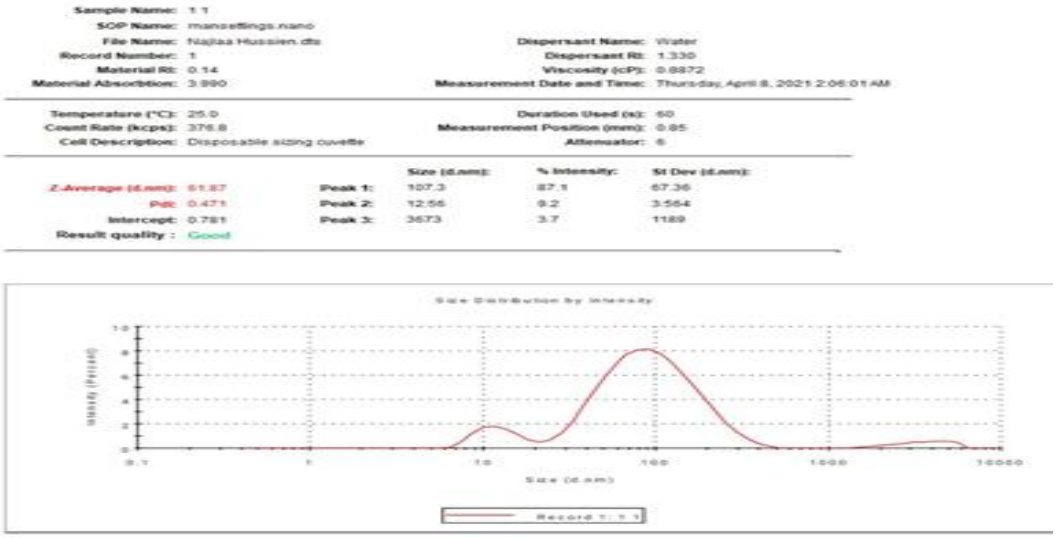

Figure 1. Measurement of the size of silver nanoparticles by zeta potential test.

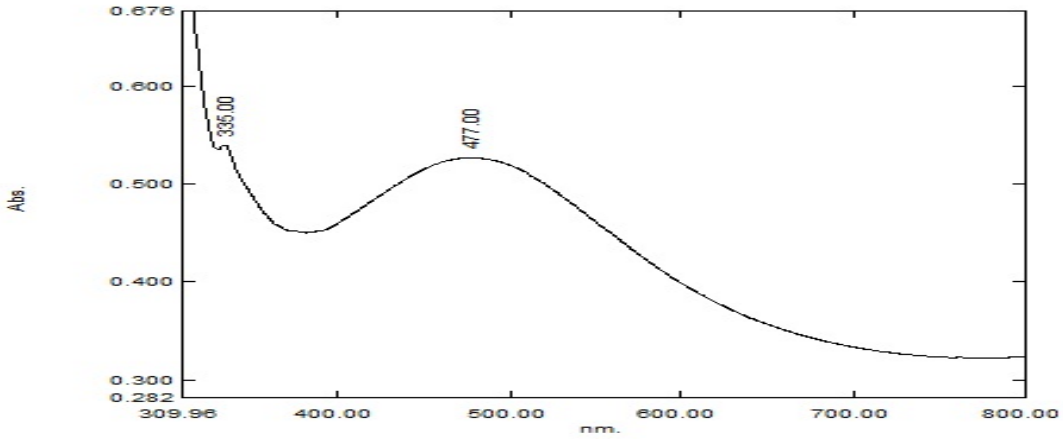

Figure 2. UV spectroscopy analysis of biosynthesized silver nanoparticles.

Silver nanoparticles concentration and fungi growth

Probing the impact of varying concentrations of silver nanoparticles on the radial growth of fungi responsible for spot and blight diseases in tomato plants ensued. The results revealed biosynthesized silver nanoparticles (AgNPs) effectively suppressed the growth of all tested fungi (Table 2, Figure 3). Notably, their potency in inhibiting the *Cladosporium* fungus surpassed other fungi, showing a significant difference with an 86.40% growth inhibition. Meanwhile, inhibition percentages for other pathogenic fungi ranged between 75.06% and 81.11%. Additionally, the experiment indicated a correlation between the effectiveness of silver nanoparticles and their concentration. The highest inhibition percentage (85.86%) was prominent at a concentration of 90 ppm, substantially outperforming concentrations of 10, 30, 50, and 70 ppm, which exhibited inhibition percentages of 72.49%, 75.49%, 78.83%, and 82.53%, respectively.

Table 2. The effect of biosynthesized nanosilver using strawberry extract in inhibiting the growth of some pathogenic fungi isolated from a tomato plant to tomato plants.

Fungi	% inhibition of the national growth of fungi					Fungi rate
	Nanoparticle silver concentrations ppm					
	10	30	50	70	90	
A. alternata	70.78	72.60	76.66	81.55	85.53	77.42
A. solani	61.88	70.00	74.11	81.55	87.78	75.06
Botrytis cinerea	69.66	73.00	74.11	77.44	81.88	75.22
C. cladosporioides	84.88	85.55	86.67	87.11	87.79	86.40
S. lignicola	75.25	76.33	82.64	85.00	86.33	81.11
Concentration rate	72.49	75.49	78.83	82.53	85.86	
$LSD_{0.05}$ Concentrations: 2.19, Fungi: 2.40, Interaction: 5.37						

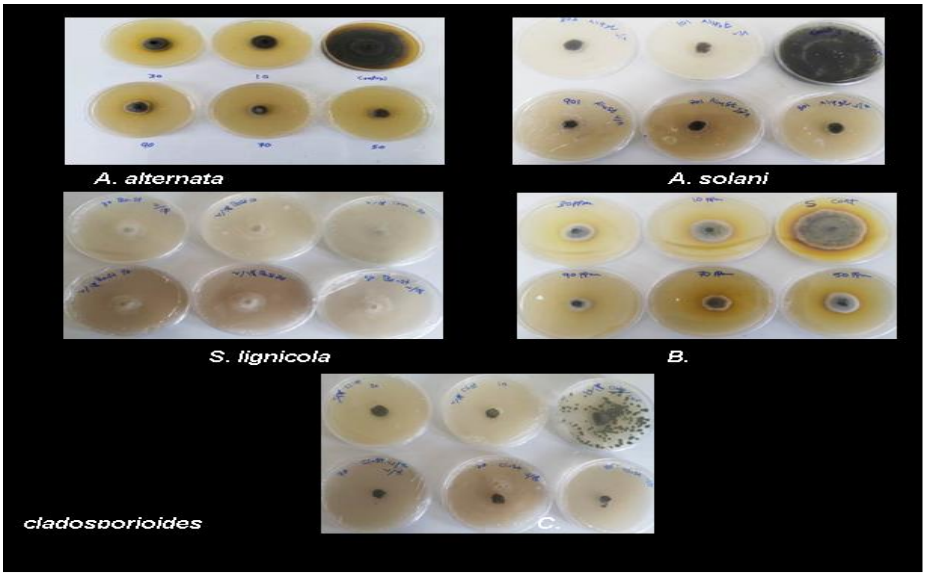

Figure 3. The effect of biosynthesized nanosilver using strawberry extract in inhibiting the growth of some tomato pathogenic fungi.

Further analysis within the same table highlighted that the highest inhibition percentages manifested in the treatment of *Cladosporium* fungus at a concentration of 90 ppm (87.79%), followed by *A. solani* fungus (87.78%). Conversely, the lowest inhibition percentage occurred at 10 ppm concentration in the *A. solani* fungus treatment, amounting to 61.88%. Previous studies corroborate these findings, citing the efficiency of silver nanoparticles in curbing various plant-pathogenic fungi. For instance, Rizwana and Alwhibi (2021) found that silver nanoparticles synthesized using mint leaf extract inhibited *Fusarium solani* growth by 63% and *A. alternata* by 61% in the culture medium**.**

Ahmed (2017) experimented with five concentrations of silver nanoparticles against *A. alternata* and *Botrytis cinerea* on bean plants. Notably, significant inhibitory effects were noteworthy at a concentration of 100 ppm, yielding inhibitions of 61.5% and 52.94% for the respective fungi. Mostafa *et al*. (2021) highlighted the inhibitory potential of silver nanoparticles synthesized using pomegranate and orange peel extracts against *A. solani*, the causative agent of early blight in tomatoes. Additionally, Ansari *et al*. (2023) reported the effectiveness of silver nanoparticles synthesized using neem leaf extract against *A. solani*. The efficacy of silver nanoparticles stems from their ability to impede fungal cell

membrane biosynthesis, disrupt plasma membrane permeability, interfere with protein biosynthesis, or hinder gene expression processes (Akpinar *et al.*, 2021).

CONCLUSIONS

This study demonstrates the potential of biosynthesized silver nanoparticles, derived from strawberry fruit extract, in effectively inhibiting the growth of various fungal pathogens responsible for spot and blight diseases in tomato (*Solanum lycopersicum* L.). The research identified 18 species of fungi associated with these symptoms, with notable efficacy against *Cladosporium*, reaching an inhibition rate of 86.4%. The experiment revealed a correlation between nanoparticle concentration and effectiveness, with the highest inhibition observed at a concentration of 90 ppm. These findings underscore the promise of nanotechnology as a sustainable and eco-friendly approach for disease control in agriculture, offering alternatives to chemical pesticides with reduced environmental impact and improved plant health outcomes. Further research and application of these findings could significantly contribute to enhancing crop productivity and sustainability in tomato cultivation.

REFERENCES

Ahmed A (2017). Chitosan and silver nanoparticles as control agents of some faba bean spot diseases*. J. Plant Pathol. Microbiol*. 8: 421. https://doi.org/10.4172/2157-7471.1000421.

Akpinar I, Unal M, Sar T (2021). Potential antifungal effects of silver nanoparticles (AgNPs) of different sizes against phytopathogenic *Fusarium oxysporum* f. sp. radicis-lycopersici (FORL) strains. *SN Appl. Sci*. 3: 506. https://doi.org/10.1007/s42452-021-04524-5.

Al-Rawi KM, Khalaf-Allah AA (1980). Design and Analysis of Agricultural Experiments. College of Agriculture and Forestry, University of Mosul. Dar-Al-Kutub for Printing and Publishing, pp. 486.

Ansari M, Ahmed S, Abbasi A, Hamad NA, Ali HM, Khan MT, Ul Haq I, Zaman Q (2023). Green synthesized silver nanoparticles: A novel approach for the enhanced growth and yield of tomato against early blight disease. *Micro-organisms* 11, 886: 1–22. https://doi.org/10.3390/microorganisms11040886.

Barman KD, Chowdhury A, Baruah PK (2020). Biosynthesis silver nanoparticles using *Zingiber officinale* rhizome extracts as efficient catalyst for the degradation of environmental pollutants. *Inorg. nano-metal chem.*, 50: 57–65.

Beer ZW, Duong TA, Barme I, Wingfield BD, Wingfield MJ (2006). Redefining Ceratocystis and allied genera. *Stud. Mycol*. 79:187–219.

Bhattacharjee J, Bhattacharjee D, Basu A, Debnath C (2018). Effect of different chemical compounds and nano-formulations on the cell wall components of *Alternaria solani* and *A. alternata*. *Indian J. Hill Farming* 31(2): 323–328.

Chaudhary AK, Yadav J, Gupta AK, Gupta K (2021). Integrated disease management of early blight (*Alternaria solani*) of potato. *Trop. Agrobiodivers.* 2(2): 77–81. http://doi.org/10.26480/trab.02.2021.77.81.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Philips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006). In phylogenetic lineages the Botryosphaeriaceae. *Stud. Mycol*. 55: 235–253.

Domsch KH, Gams W, Anderson T (1976). Compendium of Soil Fungi. Vol.1. Academic Press. London, New York, Toronto, Sydney. 859.

Ellis MB (1971). Dematiaceous hyphomycetes. Commonwealth Mycological. Kew, Surrey, England. 608.

Ellis MB (1976). More Dematiaceous hyphomycetes. Commonwealth Mycological. Kew, Surrey, England. 507.

Fayyad MA, Abbas MH (2018). Plant Diseases, Basics and Advanced (First Edition), Dar Shahryar Press, Basra, Iraq. 435.

Gupta R, Xie H (2018). Nanoparticles in daily life: Applications, toxicity and regulations. *J. Environ. Pathol Toxicol. Oncol.* 37: 209–230.

Hatem MW, Hussein HZ, Nawar MH (2021). Nanomaterial's manufactured by bacteria and plants and their role in pathogen resistance. *Ann. Romanian Soc. Cell Biol.* 25(6): 8687–8700. http://annalsofrscb.ro.

Holden PA, Gardea-Torresdey JL, Klaessig F, Turco RF, Mortimer M, Hund-Rinke K, Cohen Hubal

EA, Avery D, Barceló D, Behra R, Cohen Y, Stephan L., Ferguson P, Fernandes T, Harthorn B, Henderson W, Hoke R, Hristozov D, Johnston J, Kane A, Kapustka L, Keller A, Lenihan H, Lovell W, Murphy C, Nisbet R, Petersen E, Salinas E, Scheringer M, Sharma M, Speed D, Sultan Y, Westerhoff P, White J, Wiesner M, Wong E, Xing B, Horan M, Godwin H, Ne AE (2016). Considerations of environmentally relevant test conditions for improved evaluation of ecological hazards of engineered nanomaterials. *Environ. J. Sci. Technol*. 50: 6124–6145.

Kirankumar R, Jagadeesh KS, Krishnaraj PU, Patil MS (2008). Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen. *Karnataka J. Agric. Sci*. 21(2): 309–311.

Mostafa YS, Alamri SA, Alrumman SA, Hashem M, Baka ZA (2021). Green synthesis of silver nanoparticles using pomegranate and orange peel extracts and their antifungal activity against *Alternaria solani*, the causal agent of early blight disease of tomato. *Plants* 10: 2363. https://doi.org/10.3390/plants10112363.

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW (2013). The Botryosphaeriaceae: Genera and species known from culture. *Stud. Mycol*. 76(1): 51–167. https://doi.org/10.3114/sim0021.

Pitt JI, Hocking AD (2009). Fungi and Food Spoilage. 3rd Edition, Springer Dordrecht Heidelberg London New York Cambridge, pp. 519. https://doi.org/10.1007/978-0-387-92207-2.

Planning and Follow-up Department (2020). Basra Directorate of Agriculture-Iraqi Ministry of Agriculture-Iraq.

Rai M, Ingle AP, Paralikar P, N–Anasane RG, Ingle P (2018). Effective management of soft rot of ginger caused by *Pythium* spp. and *Fusarium* spp: Emerging role of nanotechnology. *Appl. Microbiol. Biotechnol*. 102: 6827–6839.

Rizwana H, Alwhibi MS (2021). Biosynthesis of silver nanoparticles using leaves of *Mentha pulegium*, their characterization, and antifungal properties. *Green Process. Synthe*. 10: 824–834.

Shaaban A, Al-Mallah NM (1993). Pesticides. Dar Al-Kutub for Printing and Publishing, University of Mosul. 520.

Shang Y, Hasan MK, Ahammed GJ, Li M, Yin H, Zhou J (2019). Applications of nanotechnology in plant growth and crop protection*:* A review*. Mol*. 24: 2558. https://doi.org/10.3390/ molecules24142558.

Trigiano RN, Windham MT, Windham AS (2004). Plant Pathology. Concepts and Laboratory Exercises. CRC Press LLC, Florida, USA. Protection*:* A review. *Molecules* 24: 2558. [CrossRef].

Umoren SA, Nzila AM, Sankaran S, Solomon MM, Umoren PS (2017). Green synthesis, characterization and antibacterial activities of silver nanoparticles from strawberry fruit extract. *Pol. J. Chem. Technol*. 19(4): 128–136. https://doi.org/10.1515/pjct-2017-0079.

Van der Waals JE, Korsen L, Aveling TAS (2001). A review of early blight of potato. *Afr. Plant Prot*. 70: 91–102.

Woudenberg JHC, Groenewald JZ, Binder M, Crous PW (2013). *Alternaria* redefined. *Stud. Mycol*. 75(1): 171–212.

Ying S, Guan Z, Ofpegbu PC, Clubb P, Rico C, He F, Hong J (2022). Green synthesis of nanoparticles: Current development and limitations. *Environ. Technol. Innov*. 26: 1–20.