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RESEARCH PAPER

Biosynthesis of Silver Nanoparticles for Combating Foodborne Fungi *Penicillium Digitatum* and *Aspergillus Flavus*

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ABSTRACT

Silver nanoparticles biosynthesized from the plant *Quercus infector (Q. infector*) were tested to evaluate its antifungal potential as a growth inhibitor against some fungi species named *Penicillium digitatum (P. digtatium)* and *Aspergillus flavus (A. flavus)* isolated from foodstuffs in the local market of Al-Najaf Province/ Iraq. The results stated that the highest inhibition rate was observed for *P. digitatum* at 3 mM concentration (90.97±1.72%), while the inhibition percentages for 1 mM (83.29±1.05%) and 2 mM (85.44±1.23%), indicating a concentration-dependent trend. In regards to *A. flavus,* the findings showed that the highest inhibition percentages were observed at 3 mM concentration (89.36±0.57%), while for 1 mM (73.7±1.69%) and 2 mM (85.33±0.91%), showing a similar trend. The mean percentage inhibition across all fungi was highest at 1 mM (84.24%) compared to 2 mM (79.90%) and 3 mM (75.79%). These results emphasize the antifungal efficacy which increased with the concentration of silver nanoparticles, with *P. digitatum* showing higher sensitivity compared to *A. flavus*. The results also indicate that fungal species treated without nanoparticles showed significantly lower inhibition rates (76.29% and 77.77% for *Penicillium* sp. and *Aspergillus* sp., respectively, without nanosilver treatment). The silver nanoparticles were characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR).

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INTRODUCTION

The plant species *Q. infector* is abundant in nature, and many have great potential for producing nanomaterials. These medical plants have advantages for the synthesis of nanoparticles in terms of their easy availability, safe handling, and possession of a large variety of active agents from silver ion reduction. It is considered a superior method compared to conventional chemical and physical methods due to its cost-effectiveness, energy efficiency, and safety because it is economical, energy-efficient, cost-effective, and it protects human health and the environment its products are safe to use, hence they use plant extracts, which have a huge impact on the distant future [1]. The status of working on plants' physiological and structural responses to environmental stressors, can deliver a valued understanding of their resilience and adaptability that lead to underscore the potential of plants

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as sustainable resources for applications like nanomaterial synthesis [2] by identifying stress-tolerant species with unique biochemical properties [3]. *Q. infector* is a small tree or a shrub belonging to the Fagaceae (Quercaceae) family. Galls of *Q. infector* (QI) is known by different vernacular names, locally known as If as. The plant is found in Turkey, Syria, Persia, Cyprus, and Greece [4]. Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth-regulating chemicals produced by insects or mites [5]. The galls of *Q. infector* are produced by the insect *Cynipsquer cufolii*, for depositing its eggs [6]. The Gall of this plant is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, stomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, wound healing and many other medical applications [7]. The main constituents found in the galls of *Q. infector* are (50-70%) tannin known as gall tannic acid which is a complex mixture of phenolic acid glycosides varying greatly in composition, and a small amount of free Gallic acid and ellagic acid [8].

Nanoparticles have established effectiveness in numerous medical and biological applications, including drug and vaccine delivery [9], antibacterial biocontrol agents [10-12], antimicrobial biofilm [13], antioxidants [14], and chemotaxis studies [15]. This research underlines the developing scope of nanoparticle-based systems for improving human health. Artificial intelligence (AI) has appeared as an influential tool in advancing nanotechnology, allowing precise design, synthesis, and application of nanoparticles [16]. Progressions in nanotechnology have permitted precise control over the outer surface properties of the nanomaterials at the nanoscale in order to be more effective in their applications [17]. Biosynthesized nanoparticles, particularly silver nanoparticles, have gained attention for their remarkable antimicrobial activity. The current research aims to assess the antifungal potential of silver nanoparticles AgNPs biosynthesized (eco-friendly) using the plant *Q. infector* in contradiction of two fungal species, *P. digitatum,* and *A. flavus*, isolated from some food available in the local market of Al-Najaf Province, Iraq. Precisely, the study aims to evaluate the growth inhibition efficacy of these silver nanoparticles at variable concentrations (1 mM, 2 mM, and 3 mM) and recognize concentration-dependent trends in their antifungal action. Moreover, the study comparations the sensitivity of both fungal species to nanosilver treatment, with an emphasis on decisive the higher susceptibility of *P. digitatum* compared to *A. flavus*. Comprehensive characterization of the biosynthesized nanoparticles was performed using advanced analytical techniques to prove their properties and effectiveness. The results contribute to advancing eco-friendly antifungal policies with potential applications in food preservation and fungal contamination management.

MATERIALS AND METHODS

Samples collection and plant extract preparation

Parts of dried medicinal plants were purchased from the local markets of the city of Al-Najaf Province, Iraq which is the fruit of tannins that were examined in the graduate laboratory of the Faculty of Science, University of Kufa. The samples were cleaned, and foreign materials were removed. The plants were then washed carefully with distilled water as the plants may be contaminated with many microbial species [18] or dust. Then, it was sieved to remove any liquids, then left to dry for several days at room temperature without any moisture. The cleaned-dried plants were ground using an electric mill to obtain a fine powder and then stored in sterile and airtight containers at 4°C. To prepare the plant extract, 40 g of *Q. infector* powder was taken and the volume was adjusted to 200 ml using distilled water and placed at a temperature of 80C° for homogeneity of the solution. After that, the solution was filtered by filter paper. The filtered solution was centrifuged at a speed of 10,000 rpm for 10 minutes to obtain the supernatant solution which is the required *Q. infector* extract, while the precipitate was discarded [19]. The obtained extract is used later as a reducing agent for the synthesis of silver nanoparticles [20].

Biosynthesis of Silver Nanoparticles

The ability of the *Q. infector* plant extract to produce silver nanoparticles was examined by mixing 4ml of *Q. infector* aqueous extract solution and completing the volume to 5.5ml of distilled water. Silver nitrate $AgNO_3$ at a concentration

of 1mM was added to the previous solution. The mixture was then put on a heat sink at a temperature of 90°C for two hours and waited until the color changed. The reaction mixture began to change color at 60°C followed by the brown color [21]. The silver nanoparticles were characterized by UV visibility, scanning electron microscope (SEM), atomic force microscope (AFM), X-ray diffraction, and FTIR.

Study the effect of some parameters on the synthesizing process of nanoparticles

The reaction mixture was transferred into a beaker and subjected to heating, the temperature was monitored through the thermometer, and the solution was left to experience the heating period using a magnetic stirrer. The color intensity increased with temperature, and as the reaction progressed, the color changed from dark yellow to brown over time. This indicated the initiation of particle formation, which was first observed at 60°C, with the temperature then maintained at 90°C to complete the reaction [22]. Different volumes of *Quercus infector* extract (10 mL, 20 mL, 30 mL) were tested at 90°C with a silver nitrate concentration of 1 mM, and the reaction

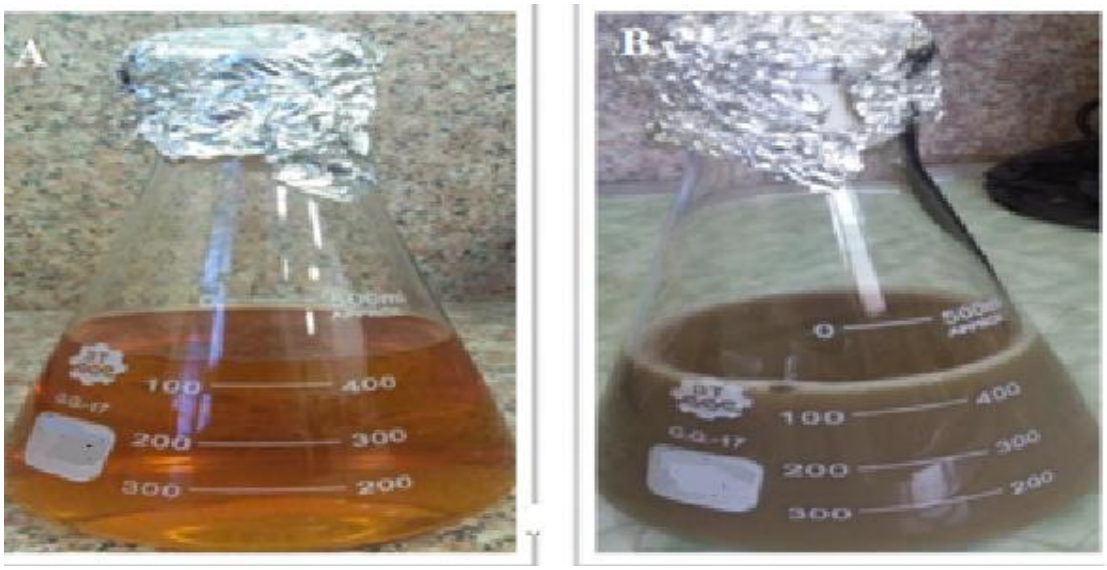

Fig. 1. (A) *Q. infector* extract before producing Ag NPs (B) *Q. infector* extract after producing Ag NPs.

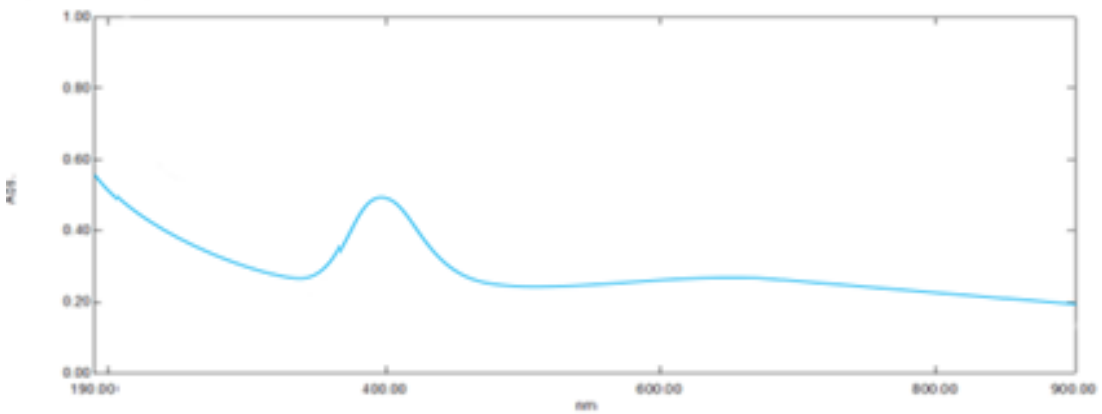

Fig. 2. UV Visible assay of biosynthesized Ag NPs by *Q. infector.*

was allowed to stabilize for two hours. The color change was monitored over time to determine the most suitable volume for preparing silver nanoparticles [23]. Different concentrations of silver nitrate (1, 2, and 3 mM) were evaluated, with the color change tracked over time to identify the optimal concentration for the synthesis of silver nanoparticles. The reaction mixture was incubated at 90°C for a duration of two hours to stabilize the process [24]. The impact of pH on silver nanoparticle synthesis was tested at pH 3, 5, and 7, using 1 mM silver nitrate and 20 mL of *Quercus infector* extract. The reaction was conducted at 90°C for two hours to determine the optimal pH [25].

Evaluation of the antifungal efficacy of AgNPs

The antifungal activity of the aqueous plant extract and synthesized silver nanoparticles was assessed using the Sabouraud Dextrose Agar (SDA) medium. For each concentration (1, 2, and 3 mM), 0.1 mL of the extract was placed on the surface of the medium, followed by the addition of a 5 mm diameter disc, taken from each fungal culture using a sterile cork borer. The Petri dishes were incubated at 25 ± 2°C for 7 days. The

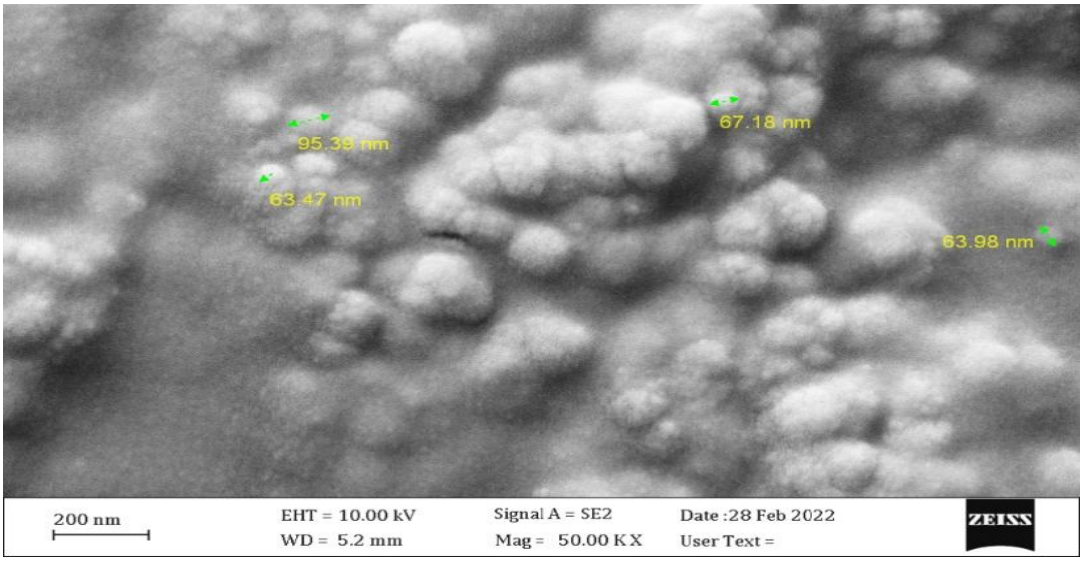

Fig. 3. FESEM Ag NPs synthesis by *Q. infector.*

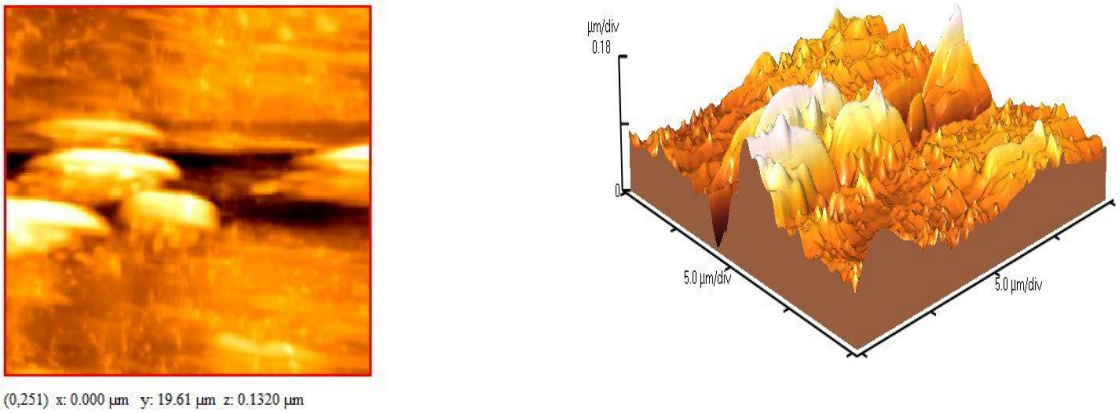

Fig. 4. AFM assay of Ag NPs.

antifungal activity was determined by measuring the inhibition zone diameter in millimeters using a ruler.

RESULTS AND DISCUSSION

The ability of the *Q. infector* extract to produce silver nanoparticles was tested by mixing the *Q. infector* extract with silver nitrate $AgNO_3$ at a concentration of 1 mM and the color began to change until became brown as shown in Fig. 1.

Fig. 2 shows the peak value taken, and the particle size was measured in nanometers by measuring the absorbance of the silver nanoparticles in the figure below and found to be about 400 nanometers.

The results showed that the synthesized silver nanoparticle surfaces exhibit a good stability and higher organization in the form of crystals with high agglomeration. As shown in Fig. 3, it is clear that the surface has reached a lower degree of crystallinity, and this may be due to increased agglomeration, furthermore, the activity will increase due to the appearance of plasmon resonance. The size readings ranged from 63.47 to 95.39nm.

Fig. 4 showed the atomic-force microscopy

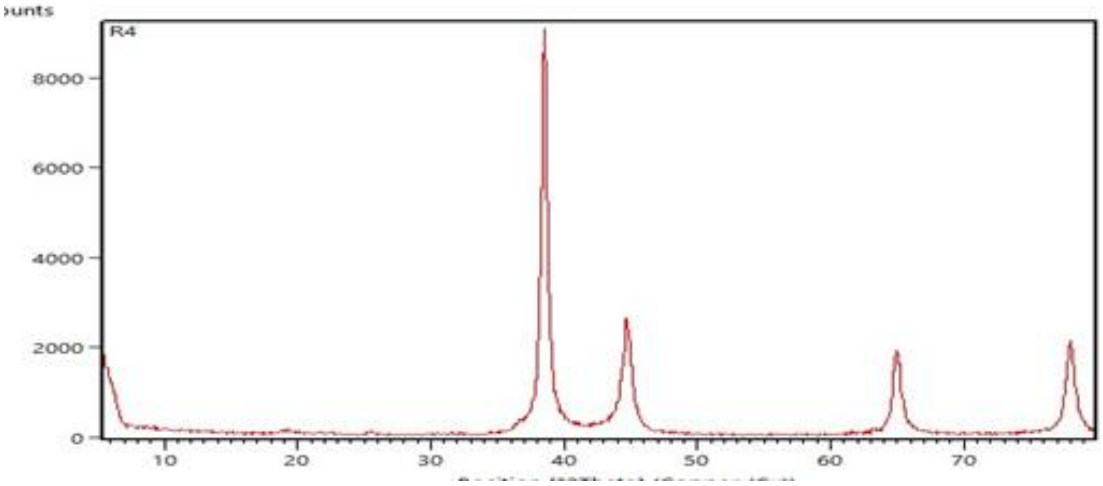

Fig. 5. XRD assay of Ag NPs

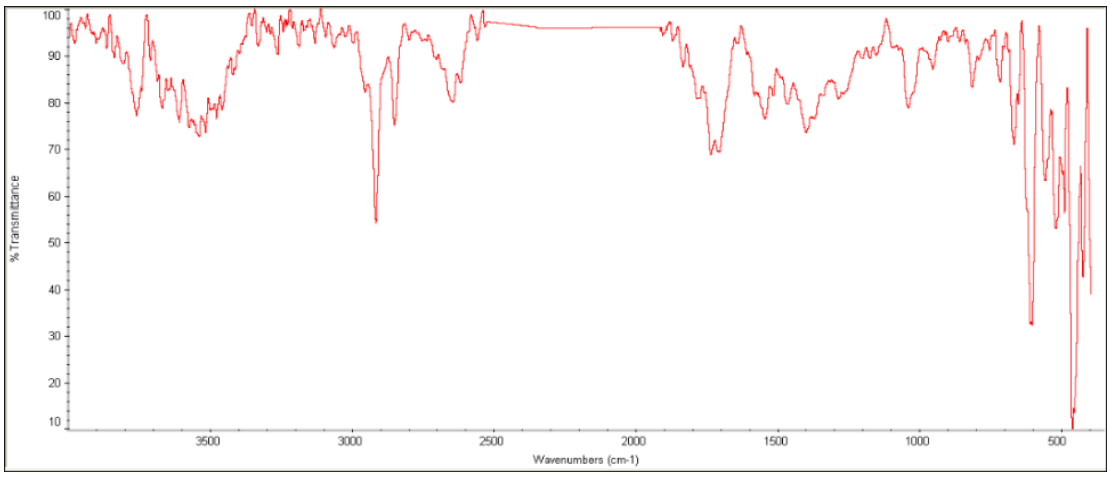

Fig. 6. FTIR assay of Ag NPs

(AFM) results conducted on green-synthesized silver nanoparticles using *Q. infector*: 3D image of silver nanoparticles analyzed by NOVA-TX software was also created on the nanoparticles, showing the particle size distribution, the size ranged from 32 to 98 nm.

X-ray diffraction spectra of the biosynthesis of silver nanoparticles from plant *Q. infector* show that the size of the silver nanoparticles is 38 nanometers as shown in Fig. 5.

Table 1. The effect of different concentrations of silver nanoparticles/ *Q. infector* extract on the percentage inhibition of diameter growth (PIDG)% of *P. digitatum* and *A. flavus*

Concentration / Type of fungi	Mean of PIDG (%) ± S. D			
	1(mM)	2(mM)	3(mM)	*Mean (b)*
Penicillium digitatum	83.29 ±1.05*	85.4±1.23*	90.97±1.72***	86.5*
Penicillium Sp.+ Q. infectoria without Nanosilver	74.44*±1.08*	75.55*±2.73*	78.88*±1.50*	76.29
Asp. Flavus	73.7±1.69	85.33 ± 0.91*	89.36±0.57**	82.79
Asp. flavus+ Q. infectoria without Nanosilver	71.11*±1.56*	73.33*±3.3*	74.07±1.06	77.77
Mean (a)	84.24**	79.90*	75.79	
Control	NI	NI	NI	
L. S. D	a= 1.614	ab= 2.2	b = 1.318	

Data are means of three replicates (n=3) ± standard deviation. NI: no inhibition. Data is presented as highly significant***, slightly significant** , and significant* at $p \leq 0.05$ within different extract concentrations. LSD was applied to all the data.

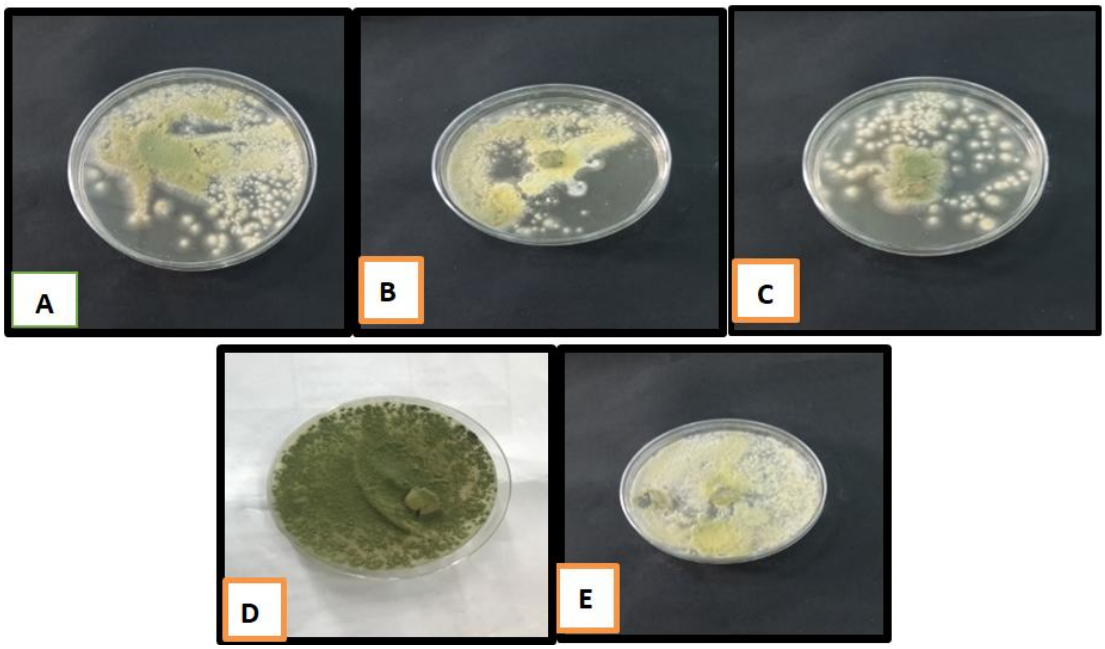

Fig. 7. Effect of different silver nitrate concentrations on the average inhibition diameter of *A. flavu,* A-1mM, B-2 mM, C-3mM, D- Q. *infector,* and E- Control treatment.

The FT -IR spectroscopic study confirmed that the carbonyl group from amino acid residues and peptides of proteins has a stronger ability to bind to metal (Fig. 6). So, the proteins could most possibly form a coat covering on the metal nanoparticles (silver nanoparticles) to prevent agglomeration of the particles and stabilize them in the medium

Table 1 shows that all concentrations of silver nitrate $AgNo_3$ used in the current study (1, 2, 3mM) and at different temperatures gave a significant and clear inhibition in the growth of the two fungi *P. digitatum* and *Asp. flavus*. the highest rate of inhibition of the two fungi was at a concentration of 3 mM. The average colony diameter of the fungus was (90.97 ± 1.72) % and (89.36 ± 0.57) % compared to the control treatment, where the average diameter of the colony was (0) %, while the rest of the concentrations (1 and 2) were the average diameter of the inhibition (1.05 ± 85.4 ±1.23, 73.7±1.69 and 85.33 ±0.91) % respectively. While the rate of inhibition of the fungus appeared *P. digitatum* and *Q. infector* without Nano silver at a concentration of 3 mM, it reached (78.88) % and *Asp. flavus* and *Q. infector* without Nano silver the percentage of inhibition in one treatment reached (74.07) % at the same concentration when comparing the results of inhibition. The best concentration at the level of the two fungi when comparing the results of inhibition was (3) mM for all concentrations, as *P. digitatum* excelled. more sensitive where it gave the highest value of significant inhibition (90.97±1.72) as illustrated in Figs. 7- 9.

The values are reported as mean ± standard deviation (SD) (n = 3), significance at $p \leq 0.05$ by two-way ANOVA, N.S: not significant

A study conducted by Luceri et al., 2023 [26], showed the relationship of each participant between the concentration of silver nitrate and the effectiveness of the nanomaterial, that is, the higher the concentrations of silver nitrate, the more inhibition of the nanomaterial increased. There was a positive correlation between the increase in silver nitrate salts and the increase in the production of nanoparticles. The antimicrobial effect of silver nanoparticles has been tested extensively. This activity is attributed to many factors that are summarized. The small size of the nanoparticles and the increase in the surface area provide greater opportunities. With bacterial and fungal cells because it leads to an increase in the permeability of the membranes and the destruction of microbial cells, silver nanoparticles can cause cell destruction and changes in the

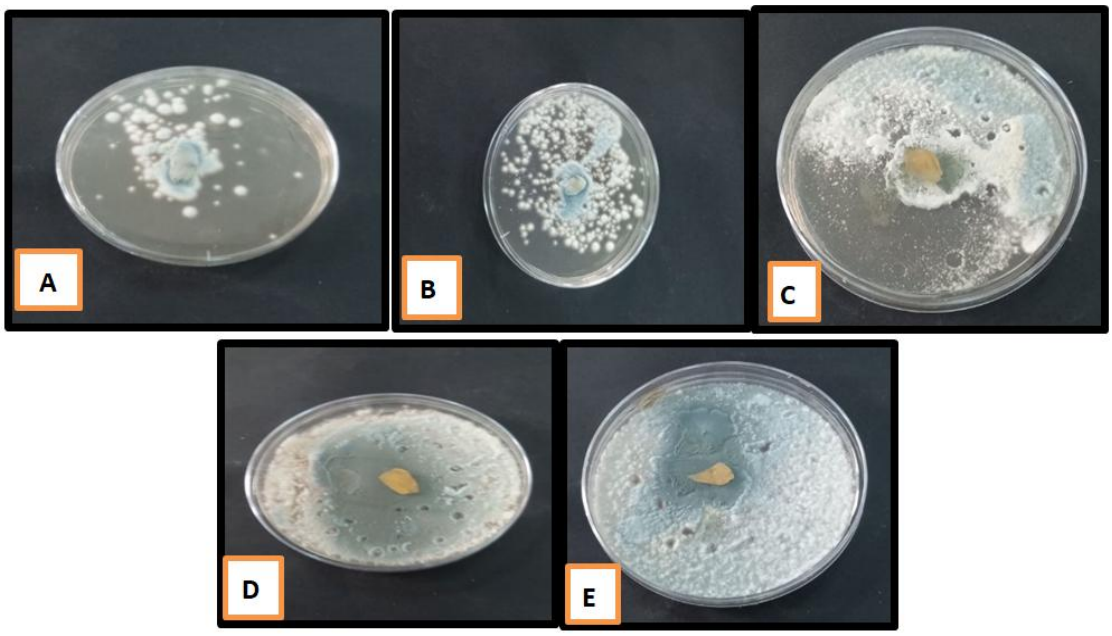

Fig. 8. Effect of different silver nitrate concentrations on the average inhibition diameter of *P. digitatum*: A-1mM, B-2mM, C-3mM, D- *Q. infector,* and E- Control treatment.

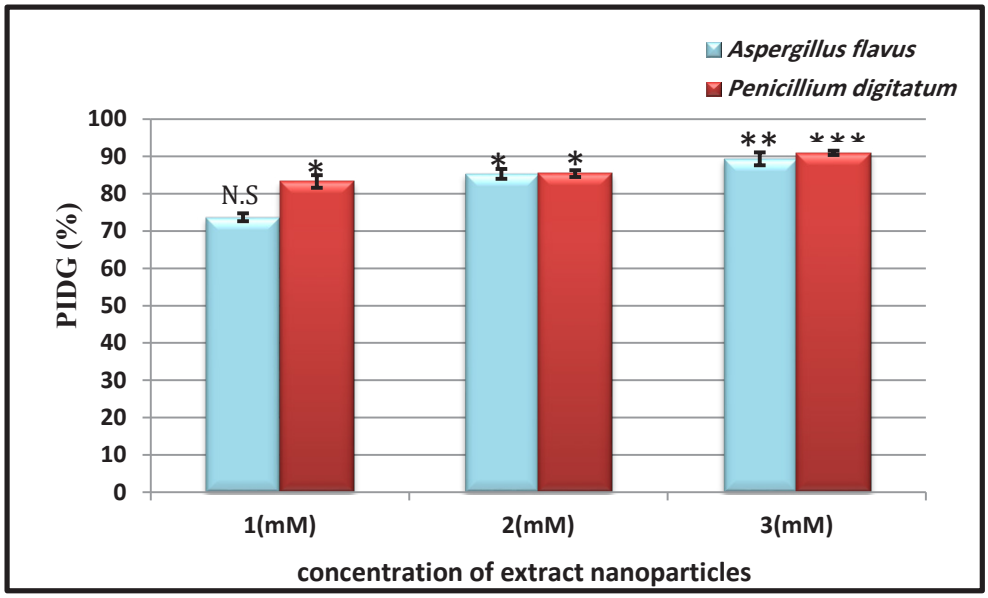

Fig. 9. Comparison of the inhibitory effect of silver nanoparticles using different concentrations.

permeability of the cell membrane, in addition, silver nanoparticles adhere to the surface of the cell membrane.

The silver nanoparticles interact with amino acids and enzymes, the researchers confirmed through the SEM scanning electron microscope that silver nanoparticles stick to the cell membrane and may penetrate the fungal cell because silver nanoparticles have a very large area compared to their small size, the Ag^+ ion In the case of silver can They are deposited in interaction with the components of the growth medium or fungal hyphae, and therefore the area of contact with microorganisms such as bacteria or fungi is large, and the possibility and ability to enter penetration of microbial cells increases. Some researchers mentioned that green silver nanoparticles manufactured from some plants such as *Calatropis procera* [27], *Lawsonia inermis* [28], *Phyllanthus amarus* [29], *Tinospora cordifolia* [30], *and Withania somnifea* [31] showed antifungal activity, the actual mechanism behind the antifungal activity of silver nanoparticles is not yet fully understood, however, it is supposed to break down the structure of the cell membrane by destroying the integrity of the membrane [32]. This disruption likely increases membrane permeability, leading to cellular damage and ultimately inhibiting fungal growth. Such findings underscore the potential of plant-based silver nanoparticles as effective antifungal agents, while also highlighting the need for further research to elucidate the molecular mechanisms underlying their activity.

CONCLUSION

The present study validates the powerful antifungal activity of silver nanoparticles biosynthesized using *Quercus infector* extract against *Penicillium digitatum* and *Aspergillus flavus*. The results exposed a concentration dependent inhibition, with *P. digitatum* exhibiting superior sensitivity compared to *A. flavus*. The maximum antifungal efficiency was detected at a concentration of 3 mM, highlighting the potential of green-synthesized nanoparticles as an eco-friendly substitute to traditional antifungal agents. Additionally, the comprehensive characterization of these nanoparticles approves their stability and suitability for biological and medical applications. These results pave the way for future research on nanoparticle based antifungal treatments, mainly in food preservation and fungal contamination control, offering a sustainable solution with minimal environmental impact.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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