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SYNTHESIS OF GREEN SILVER NANOPARTICLES USING TURMERIC EXTRACTS AND THEIR ANTIFUNGAL EFFICACY AGAINST PLANT PATHOGENIC FUNGI

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SUMMARY

This study demonstrates the biosynthesis and characterization of silver nanoparticles (AgNPs) using aqueous and alcoholic extracts of turmeric (*Curcuma longa*). The synthesized AgNPs entailed characterization by UV-Vis spectroscopy, atomic force microscopy (AFM), and Fourier-transform infrared spectroscopy (FTIR). The UV-Vis analysis revealed absorption peaks between 300 and 520 nm, confirming nanoparticle AFM indicated nanoparticle size ranges of 0–23 nm for the alcoholic extract and 1–10 nm for the aqueous extract. The FTIR analysis identified the compounds responsible for reducing the silver nitrate. The antifungal activity measurement of the synthesized AgNPs ensued against three phytopathogenic fungi: *Alternaria alternata*, *Alternaria dianthi*, and *Fusarium verticillioides*. The results demonstrated silver nanoparticles effectively inhibited fungal growth, with an inhibition zone diameter of 52.4 mm in *A. alternata*, 52.0 cm in *A. dianthi*, and 41.5 mm in *F. verticillioides*. Treatment with biosynthesized AgNPs also resulted in reduced fungal biomass and elevated pH levels in the culture medium. The effectiveness of bio-silver nanoparticles against *A. alternata, A. dianthi, and F. verticillioides* fungi succeeded in testing at different concentrations. It further showed the highest inhibitory concentration (20 ppm) for all the fungi, averaging over colony diameters (3.1, 3.9, and 1.5 cm, respectively) for nanoparticles of the aqueous extract and fungal colony diameters (2.3, 3.1, and 2.2 cm) in the alcoholic extract.

Keywords: Silver nanoparticles, aqueous and alcoholic turmeric extracts, antifungal activity, fungal biomass, pH level, UV-Vis, FTIR, AFM

Key findings: Silver nanoparticles produced from turmeric plant extracts proved to be a safe antifungal substance and highly effective in inhibiting the growth of fungi *A. alternata*, *A. dianthi,* and *F. verticillioides.*

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INTRODUCTION

Pathogenic fungi pose a significant threat to both human health and agricultural productivity, representing a complex challenge with far-reaching implications (Zouari *et al*., 2016; Farian and Wójcik-Fatla, 2022). Phytosanitary fungi are a group of microorganisms showing various mechanisms of infection, and the variations in infection methods help them to infect numerous crops (Al-Taee, 2010; Qassim *et al*., 2023). Fungi are a plant's injury factor and cause significant losses in crop plants. Fungi treatment usually uses different biological and chemical methods, such as fungicides, as well as several field, medicinal, and ornamental plant additives (Mirkova and Konstantinova, 2003; Qassim *et al*., 2024).

Farmers use agricultural pesticides, and with the evolution of modern science and application methods for pest control, the latest research has progressed on using nanomaterials, a safe and environmentally friendly pest control method. These substances also degrade easily in the ecosystem and have no side effects. Additionally, it is difficult for pests to form resistant strains rapidly (Fahim *et al.,* 2024; Almashhadani and Qassim, 2025a).

The Japanese scientist, Norio Taniguchi, first identified NATO's (North Atlantic Treaty Organization) technology in 1974 as a set of atomic and molecular material separations, compositions, and mergers (Cortez *et al*., 2011). Nanoparticles (NPs) have a considerably more significant role in the management of plant diseases compared with manufactured fungicides. The nanoparticles have demonstrated an inhibitory effect on microorganism growth and survival (Rico *et al*., 2011; Alzubaidi *et al*., 2023).

Nanotechnology has made tremendous achievements over the past decades, particularly between 2005 and 2010. Its evolution has obviously increased and even doubled in comparison to previous decades in the number of products containing and requiring nanoparticles to produce them. These developments have received support from their unique general properties, specifically in the particle size, wide surface area, high chemical stability, surface interaction, charge, and shape for their regular-sized counterparts (Nawabjohn *et al.,* 2022; Elazab *et al.,* 2024).

Recently, growing interest has surfaced in the applications of nanotechnology in agricultural systems, particularly through the use of nanoparticles composed of semi-metals and metal oxides with dimensions ranging from one to 100 nanometers. This ascribes to their small size, large surface area, and their interaction, enabling them to be used as effective fungicides and nanoparticles, which can alleviate the current challenges in disease management by reducing inputs (Shahzaib *et al*., 2024; Almashhadani and Qassim, 2025b). Therefore, the following study aimed to synthesize and characterize the silver nanoparticles (AgNPs) using aqueous and alcoholic turmeric extracts.

MATERIALS AND METHODS

The isolates

Three fungal isolates belonging to the fungal species *A. alternata, A. dianthi,* and *F. verticillioides* underwent procurement from the isolates bank at the Department of Biology, College of Science, University of Mosul, Mosul, Iraq. All the fungal isolates already have diagnoses in previous studies.

Culture media

The medium based on potato dextrose agar (PDA) incurred preparations according to the protocol provided by the manufacturer (HiMedia, India), with the addition of the antibiotic streptomycin at a concentration of 100 mg/L (Al-Taee, 2010; Al-Healy and Al-Taee, 2023).

The preparation of the potato-dextrose-broth (PDB) medium comprised mixing 200 g of peeled potato extract with dextrose, complementing the size to one liter with distilled water, setting the pH at 6, and sterilizing (Mohamed *et al*., 2019; Hmood and Qassim, 2023).

Preparation of silver nanoparticles

The commercial silver nanoparticles (AgNPs) came from the US Research Nanomaterials and have the following characteristics (CAS number = 7440-22-4, 50 nm, assay 99.5%, and MW = 107.87). In the antifungal activity of AgNPs, the three different concentrations (50, 100, and 200 ppm) entailed preparations by dissolving 5, 10, and 20 mg, respectively, of AgNPs in 2 ml of solvent (7 hexane: 3 ethyl acetate) before completing the size of 100 ml with deionized distilled water.

Effectiveness of AgNPs in PDA

The effectiveness of AgNPs preceded its testing against the studied isolates using the concentrations of 50, 100, and 200 ppm. The center of the plates reached inoculation with a disc of 5 mm taken from the edge of a pure fungal culture at the age of five days. The petri dishes sustained incubation at the temperature of 25 °C ± 2 °C for six days with daily follow-up (Kim *et al*., 2012).

$$\text{Percentage inhibition} = \frac{\text{control} - \text{treatment}}{\text{control}} \times 100$$

Effectiveness of AgNPs in PDB

The silver nanoparticle activity also involved testing against the studied isolates in the PDB medium in three ways—PS medium with a solvent (ethyl acetate: hexane 3), PS with silver nitrate, and PS with silver nanoparticles. Previously prepared medium with AgNPs ppm concentrations (50, 100, and 200 ppm) reached inoculation with fungi, incubated for seven days at 25 °C, and then filtered the biomass. Consequently, it entailed drying at 70 °C for 24 h before weighing to assess the inhibition effectiveness.

Preparation of bio-silver nanoparticles for turmeric

For the preparation of aqueous nonionic extract and alcoholic extract of the turmeric plant, the turmeric roots attained cleaning, grinding, and extraction to prepare 5% solutions in distilled water and 96% ethanol. The mixtures bore shaking at 40 °C for 30 min before filtering and storing at 4 °C for future use.

For the fabrication of bio-nanoparticles of silver, the turmeric silver bioparticles' synthesis included mixing 10 ml of the turmeric extract with 100 ml of 1 mM silver nitrate, heating at 45 °C for 20 min, and observing the color change. The solution entailed filtration and storage in light-protected bottles for five days, with the control prepared similarly (Nawabjohn *et al.,* 2022).

Characterization of silver nanoparticles

The UV/VIS-visible absorption spectrometer served to detect the optical properties of the raw extract of the turmeric plant and the silver nanoparticles manufactured from it at wavelengths of 200–1100 nm. The absorbency measurement of the dissolved solutions continued in 3% ethanol, with the turmeric dissolved in water by 3% as well. Taking a sample of the bio nanoparticles after 48 h of preparation gained examination at room temperature (Ponarulselvam *et al.,* 2012).

For Fourier transform infrared spectroscopy (FTIR), the produced silver-nano solution and potassium bromide attained mixing in the ratio of 1:100 before scrutiny using the FTIR spectrometry within the range of 400–4000 cm^{-1} to study the molecular vibrations of the sample molecules. The importance of this examination lies in knowing the elements in the sample, with the spectra measured at room temperature. All these analyses transpired at the Department of Materials Research, Ministry of Science and Technology, Iraq (Alzubaidi *et al.,* 2023).

On the AFM (atomic force microscope), a biosynthetic silver Nano solution received drying on a glass slide before being examined with an atomic force microscope to analyze surface topography, roughness, and grain size (Logeswari *et al*., 2015).

RESULTS AND DISCUSSION

Effect of silver nanoparticles in the solid media

Results showed the efficiency of silver nanoparticles in inhibiting the studied pathogenic fungal isolates on the PDA culture, causing high inhibitory efficiency with three concentrations (50, 100, and 200 ppm) compared with the control treatment (Table 1) (Figure 1). The fungal isolates *A. alternata, A. dianthi*, and *F. verticillioides* achieved the highest inhibition rates in the colony diameter, amounting to 5.24, 5.20, and 4.15 cm, respectively. These results also confirmed the effectiveness of silver nanoparticles to inhibit the growth of fungus. The silver nanoparticles emerged to be more influential in the medium of fungi growth due to the high surface area and small size that enables them to penetrate the cell wall easily. Thus, it leads to the rupture and decomposition of the fungal filaments and death of the fungus (Baran *et al*., 2023; Almashhadani and Qassim, 2025b).

Effect of silver nanoparticles in the liquid medium

The fungi behaved similarly in the three treatments, and the fungi showed a biomass with three concentrations (50, 100, and 200 ppm) in all additions and the comparison treatment, with the biomass unaffected by adding the solvent (ethyl acetate: hexane 3) (Table 2). The fungus *A. alternata* appeared with a biomass of 2.6 g as a comparison treatment and 1.82 g with the medium treated with silver nitrate. It gave a biomass of 2.15 g with the medium treated with silver nanoparticles, which was accompanied by an increase in the acidity function in the medium. As for the fungus *A. dianthi,* the same behavior was evident as with the previous fungus through the effect of the fungal biomass. It showed the lowest biomass (1.96 g) for the addition of silver nitrate, and 1.46 g for the addition of silver nanoparticles. For *F. verticillioides*, a biomass of 1.99 g resulted at the 200 ppm of silver nitrate, and 1.52 g in the medium with silver nanoparticles.

Increasing concentrations also raised the pH levels gradually. The inhibition may refer to the nanoparticles with small particles that can easily penetrate the cell wall and form free radicals, disrupting cell proteins by the released silver ions. After absorbing the released silver ions by the pathogenic cell, it leads to the disruption of respiratory enzymes and the generation of reactive oxygen species, thus preventing the formation of ATP (adenosine triphosphate), which further disturbs the cell membrane. It also causes modifications in the DNA and, in turn, leads to the cell's death (Almashhadani and Qassim, 2025b).

Table 1. Effect of various concentrations of Nano silver on the growth rate of pathogenic fungi.

Fungi	Concentrations	Colony diameter (mm)	Inhibition ratio (%)
A. alternata	50	57.5	2.5
	100	55.0	6.7
	200	52.4	0.001
Comparison	0	59.0	
A. dianthi	50	59.0	16.05
	100	54.8	12.3
	200	52.0	16.8
Comparison	0	62.5	
F. verticillioides	50	48.5	4.9
	100	44.5	12.7
	200	41.5	18.6
Comparison	0	51.0	

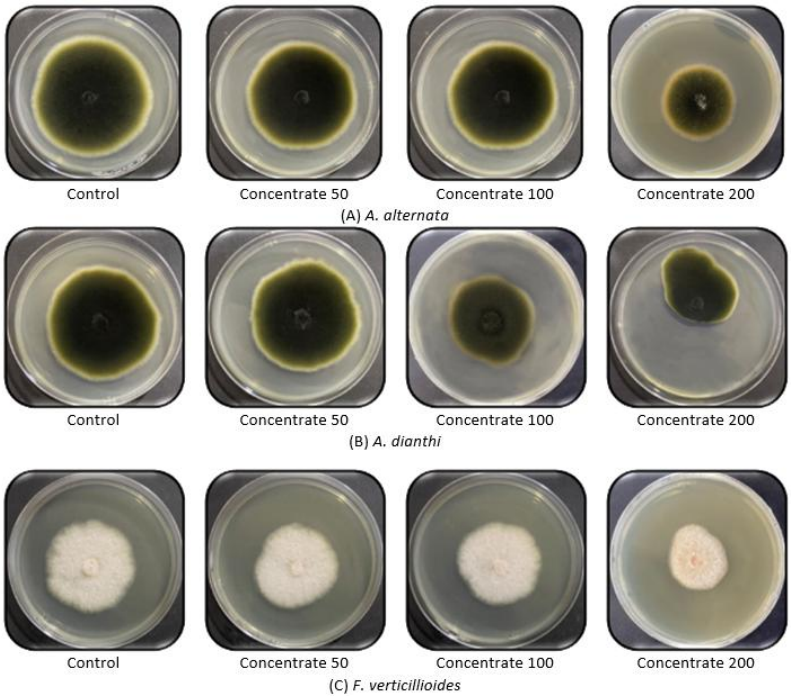

Figure 1. Effect of various concentrations of Nano silver in the inhibition of A) *A. alternata*, B) *A. dianthi*, and C) *F. verticillioides.*

Table 2. Effect of various nanoscale silver concentrations in the inhibition of pathogenic fungi in the liquid medium.

No.	Fungi	Solvent + PS			Silver nitrate + PS		Nanoscale silver nitrate + PS	
		pH	Living mass (g)	Concentrations	pH	Living mass (g)	pH	Living mass (g)
1	*A. alternata*	6.35	2.75	50	6.62	2.125	6.72	2.495
		6.1	2.87	100	6.57	2.19	6.5	2.81
		6.6	2.61	200	6.42	1.82	6.5	2.15
2	*A. dianthi*	6.5	2.88	50	6.6	2.19	6.8	1.46
		6.25	2.65	100	6.6	1.965	6.57	2.945
		6.5	2.69	200	6.4	2.5	6.55	1.87
3	*F. verticillioides*	6.15	2.11	50	6.65	2.285	6.02	1.63
		6.15	1.89	100	6.6	2.165	6.1	2.09
		6	1.72	200	6.87	1.99	6.15	1.52

Biosynthesis of silver nanoparticles

The biosynthesis of silver nanoparticles using aqueous turmeric extract showed a color change from yellow to transparent yellow (Figure 2) within 20 minutes of adding it to the silver nitrate solution ($AgNO_3$) at 45 °C. This indicates an interaction between plant active compounds and the silver nitrate. The results confirmed nanoparticle formation through reduction, driven by surface plasmon resonance, a characteristic of metals like silver at the nanoscale. The color shift serves as a visual indicator of successful biosynthesis, reflecting the transformation of metal particles into nano-sized structures (Elazab *et al.,* 2024). The different colors occurring in the colloidal solution were spherical, pentagonal, and irregular circular shapes of silver nanoparticles. Moreover, the reduction of silver nanoparticles to silver nanoparticles after treatment with plant extracts can result in a

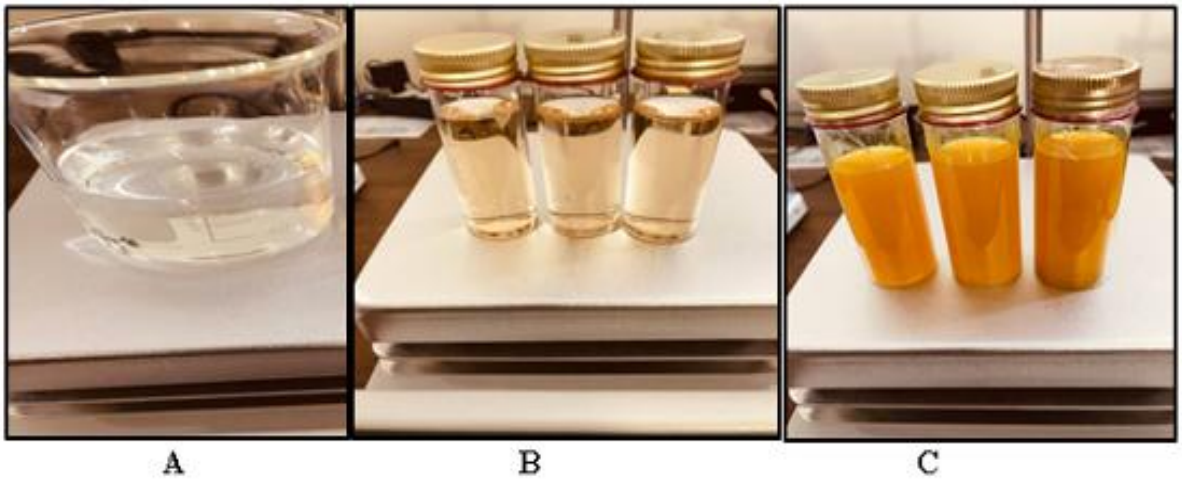

Figure 2. Color change during the nanoparticle bio-manufacturing process. A) Silver nitrate solution, B) Non-ionic aqueous extract of turmeric plant, and C) Color change of turmeric plant non-ionic extract 48 hours after silver nitrate addition and formation of silver nanoparticles.

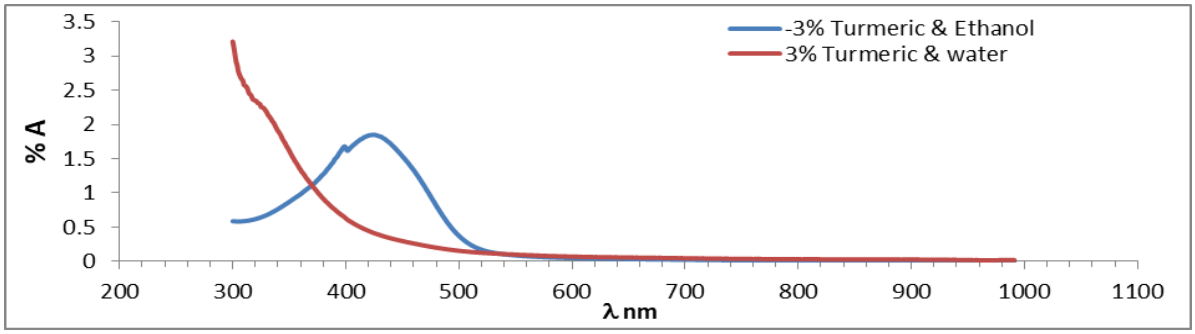

Figure 3. Examination of the UV/Vis absorption spectrum of the ionic and alcohol aqueous extraction of turmeric.

change in color (Rout *et al*., 2009). Plants contain various metabolites like triterpenoids, phenols, alkaloids, and flavonoids, which help stabilize and reduce the silver ions to AgNPs. These nanoparticles display different colors due to their optical properties, and the longer incubation times enhanced the reduction rate and nanoparticle formation, resulting in a deeper color (Vanaja and Annadural, 2013; Saliem *et al*., 2016).

Characterization of silver nanoparticles

The raw turmeric extract and its silver nanoparticles across 200–1100 nm gained analysis through UV/VIS visible absorption spectrometer. The absorbance measurement of solutions in ethanol (3%) and water (3%) revealed notable differences between 300–500 nm (Figure 3). Ethanol's absorbance considerably increased up to 430–440 nm before decreasing at 520 nm, while water's solution peak absorption was around 300 nm, and then declined toward 520 nm. The turmeric-ethanol solution with silver nanoparticles exhibited a significant reduction in absorbance between 300–520 nm, stabilizing at the lowest level across 300–1000 nm. Within the typical plasmon resonance range of 400–450 nm, the silver nanoparticles displayed an absorption peak at 440 nm. Such outcome was consistent with previous studies,

where the UV spectrometer analysis of silver nanoparticles made from ivy and *Aloe vera* extracts showed peak absorption at 419–427 nm (Tharani *et al.,* 2023).

Silver nanoparticles produced using celery Wallace exhibited peaks at 408–410 nm within the typical silver absorption range of 400–450 nm, similar to past findings. The absorption at 424 nm for silver nanoparticles obtained from orange crusts was around 390 nm (Nayak *et al*., 2020). Variations in absorbance were natural and dependent on the nanoparticle size and shape, the source of silver, and plant extract differences caused by environmental factors. Overall, the absorption significantly decreased between 300 and –1100 nm, with low values within 310–600 nm. It indicates silver nanoparticles help reduce absorption and maintain stability across this range both in water and ethanol media, likely due to interactions with turmeric.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR is a crucial technique used for analyzing surface-active aggregates, which play a crucial role in the synthesis of nanocomposites and nanoparticles. The presented study focused on the spectral range of 450–40,000 cm^{-1} to identify functional aggregates in the aqueous profiles of turmeric, which facilitate the reduction of silver nitrate to Nano silver. The prepared mixture of 4% silver nitrate and 96% KPR became molded into a specific form before its analysis. The FTIR results revealed considerable absorption bands corresponding to silver nanomaterials, particularly ranging from 1600 to 1700 cm^{-1}. Additionally, the analysis of silver nano-extracted samples dissolved in deionized water and turmeric solution displayed significant spectral features and considerable OH bonds at 3849.98 cm^{-1}, CN bands at 2358.17 cm^{-1}, and C bands at 1638.17 cm^{-1}. Furthermore, similar results were evident for Nano silver dissolved in ethanol alcohol, confirming the presence of Nano silver through distinct absorption peaks.

Atomic force microscopy test (AFM)

The use of atomic force microscopy (AFM) served to analyze the silver nanoparticles, revealing their shapes, topography, roughness, and surface protrusions through digital 2D and 3D images (Elazab *et al*., 2024; Almashhadani and Qassim, 2025a). The results enunciated that 3D surface heights ranged from 0 to 230 nm in alcohol and 0 to 10.8 nm in deionized water (Figure 4). The AFM images detailed that turmeric dissolved in ethanol produced rougher surfaces than those dissolved in water. In ethanol samples, the highest peaks ranged from 82 to 88 nm with RMS values of 20–26, and the nanoparticle size averaged between 25 and 36 nm (Table 3). In water, the peaks ranged from 34 to 71 nm with RMS values of 12–23 nm, and the particle size averaged between 15 and 30 nm. Particle size distribution indicated most particles were under 15–30 nm.

Antifungal efficacy of biogenically synthesized silver nanoparticles

The measurement of the effectiveness of silver nanoparticles prepared biologically from turmeric and alcoholic aqueous extract occurred against the fungi *A. dianthi*, *A. alternata*, and *F. verticillioides*. Results indicated morphological variations in fungal colony growth, with a decrease in growth rate as per concentrations of silver nanoparticles (5, 10, and 20 ppm) (Table 4, Figure 5). The fungi *A. alternata* showed a colony diameter of 3.1 cm at 20 ppm compared to 5.8 cm in the control. *A. dianthi* recorded 3.9 cm at 20 ppm versus 6.3 cm in the control, while the fungi *F. verticillioides* had the most significant reduction, measuring 1.5 cm against 5.2 cm in the control. The treatment with silver nanoparticles from turmeric's bio-alcohol extract also demonstrated obvious inhibitory effects, with colony diameters for the fungi *A. alternata*, *A. dianthi*, and *F. verticillioides* at 2.3, 3.1, and 2.2 cm, respectively, at 20 ppm (Table 4, Figure 6). These results suggested

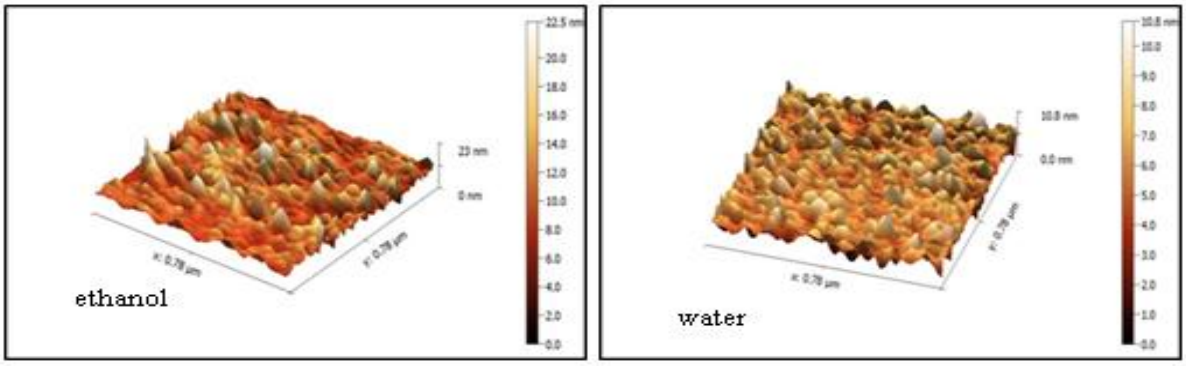

Figure 4. Two-dimensional image of silver nanoparticles by mercuric alcohol extraction with water turmeric extraction using the atomic force microscope.

Table 3. Alcohol and water top peaks using atomic force microscope.

Ethanol and water	Root-mean-square height	Maximum height	Arithmetic mean height	Average grain size
Ethanol	20–26 nm	82–88 nm	18.10 nm	25–36 nm
Water	12–23 nm	34–71 nm	10.74 nm	15–30 nm

Table *4*. Effectiveness test of biologically prepared silver nanoparticles from aqueous ionic and alcohol extract of turmeric for pathogenic fungi.

No.	Fungi's name	Bioconcentration of silver nanoparticles (ppm)	Aqueous ionic extract		Alcohol extract	
			Colonial diameter for fungus mm/day	Percentage inhibition (%)	Colonial diameter for fungus mm/day	Percentage inhibition (%)
1	*A. alternata*	Control	58.0		58.0	
		5	59.0	17.7	46.6	20.6
		10	40.0	31.03	32.0	44.8
		20	31	46.5	23.0	60.34
2	*A. dianthi*	Control	63.0		63.0	
		5	55.0	12.6	56.0	11.11
		10	47.0	25.3	39.0	38.09
		20	39.0	38.09	31.0	50.7
3	*F. verticillioides*	Control	52.0		52.0	
		5	44.0	15.3	47.0	9.6
		10	32.0	38.4	38.0	26.9
		20	15.0	71.1	22.0	57.69

silver nanoparticles effectively inhibit the filamentous fungi growth, possibly due to mechanisms as discussed in past studies (Shahzaib *et al.,* 2024).

Silver nanoparticles exert antifungal effects primarily by penetrating fungal hyphae and interacting with cell membranes. This interaction leads to the disruption of vital cellular processes, including DNA replication and protein synthesis, ultimately resulting in cell death. The nanoparticles adhere to and penetrate fungal cell walls, causing damage to proteins, lipids, nucleic acids, spores, and reproductive structures. Additionally, silver nanoparticles can influence the pathogenic DNA of fungi, impairing its replication and cloning capabilities. The silver nanoparticles also interfere with metabolic functions by affecting protein and enzyme regulation, which further inhibit fungal growth and compromise essential cellular components (Kim *et al*., 2012; Pallath *et al*., 2024).

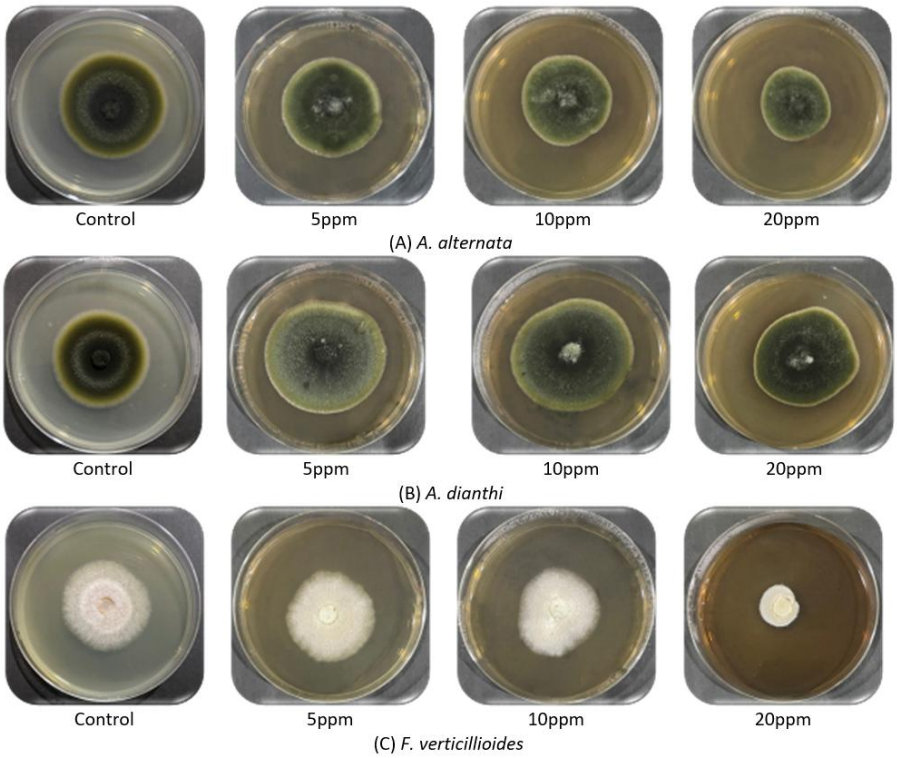

Figure 5. Effects of different concentrations of bio-silver nanoparticulate of ionic aqueous extract of turmeric on A) *A. alternata*, B) *A. dianthi*, and C) *F. verticillioides.*

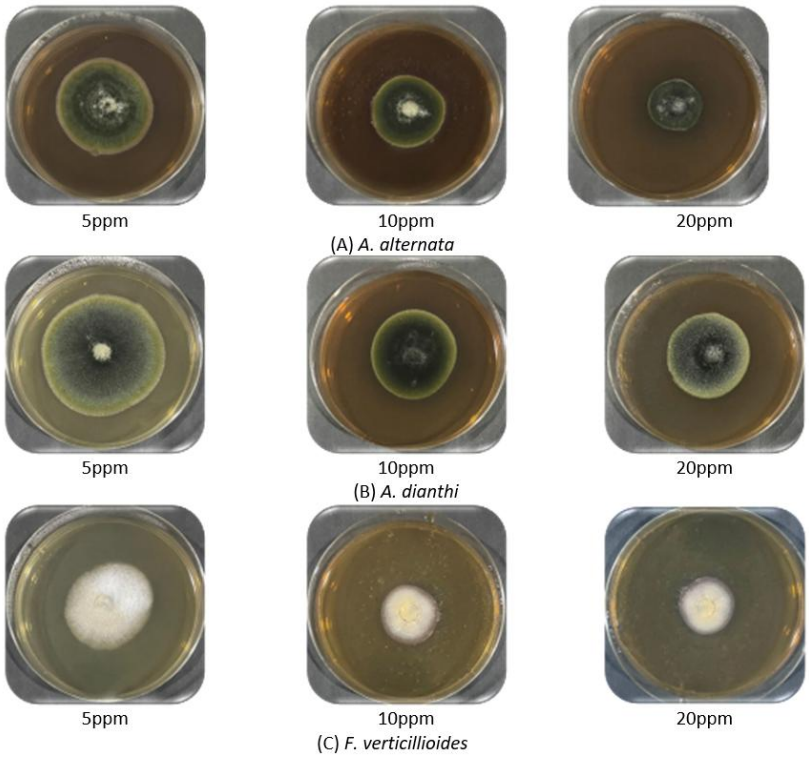

Figure 6. Effects of different concentrations of bio-silver nanoparticulate of alcohol extract of turmeric on A) *A. alternata*, B) *A. dianthi*, and C) *F. verticillioides.*

CONCLUSIONS

The study demonstrated the efficacy of silver nanoparticles against pathogenic fungi *A. alternata*, *A. dianthi*, and *F. verticillioides*. Likewise, it revealed the feasibility of bio-manufacturing silver nanoparticles using aqueous non-ionic and alcoholic solutions of turmeric, based on UV-visible spectroscopy, FTIR, AFM analysis, and color change. Moreover, the study detailed the effectiveness of nanoparticles in reducing the growth and diameter of fungi colonies. The highest inhibitory concentration was 20 ppm, achieving colony diameters of alcohol extract (2.3, 3.1, and 2.2 cm, respectively), with inhibition percentages of 60.34%, 50.7%, and 1.5%.

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