Research Article

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Corylus avellana leaf extract-mediated green synthesis of antifungal silver nanoparticles using microwave irradiation and assessment of their properties

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Abstract: Due to high antimicrobial activity against numerous microorganisms, silver nanoparticles (AgNPs) are being utilized in various areas. Microwave-accelerated AgNPs synthesis using *Corylus avellana* leaf extract was evaluated. Based on randomly central composite design, 13 mixture solutions containing different amounts of the prepared extract (0.10–0.90 mL) and 1 mM silver nitrate solution (15–25 mL) were prepared and exposed to microwave irradiation for 180 s. Response surface methodology was utilized to evaluate the effects of the two independent variables on particle size and concentration of the synthesized AgNPs, as manifested in the place of broad emission peak (λ_{max}) and its absorbance unit, respectively. Fourier transform infrared spectroscopy analysis indicated that the two hydroxyl and carboxylic acid functional groups with reducing activity existed in the prepared extract. Dynamic light scattering and transmission electron microscopy analyses revealed that the formed spherical AgNPs using optimum amounts of *C. avellana* leaf extract (0.9 mL) and 1 mM silver nitrate solution (25 mL) had minimum particle size (103.5 nm) and polydispersity index (PDI) (0.209), and maximum concentration (140 ppm) and zeta potential (–21.8 mV). Results indicated that the formed AgNPs had high fungicidal effects against the spoiled fungi of *Colletotrichum coccodes* and *Penicillium digitatum*.

Keywords: antifungal activity, *Corylus avellana*, microwave irradiation, optimization, silver nanoparticles

1 Introduction

Among the various noble metal nanoparticles (NPs), silver NPs (AgNPs) have attracted distinct attention and application in numerous industries and fields due to their unique properties, especially, their strong antimicrobial activity against various microorganisms such as bacteria and fungi strains [1–3]. In fact, AgNPs are known as a new generation of antibiotics. Due to the high surface-to-volume ratio of the AgNPs, these NPs can get easily attached to the microorganism's membrane and by releasing silver ions can change the membrane permeability and respiratory enzymes' activities, and cause cell death [4,5]. Green synthesis methods in the fabrication of metal and metal oxide NPs have several advantages compared to the synthesis techniques based on physico-chemical approaches. In fact, green or biological synthesis methods are intensified and clean, and ions reduction, NPs' formation, and their stabilization are completed in a one-step process using bioactive compounds. Furthermore, the NPs' biosynthesis procedures are cost effective and environmentally friendly because they do not use chemical solvents and reagents in their synthesis procedures [6,7]. In green metal NPs synthesis approach, many natural biomolecules of plants and their derivatives (i.e., steam, root, leaf, and flower) such as proteins/enzymes, amino acids, polysaccharides, alkaloids, phenolic, alcoholic

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compounds, and vitamins can be involved in the bioreduction, formation, and stabilization of AgNPs [8].

Hazelnut (*Corylus avellana* L.) belonging to the Betulaceae family contains high amount of fat which increases its nutritional value. Mono- and polyunsaturated fatty acids are the predominant fatty acids in hazelnut [9]. Several studies have indicated that the aqueous extracts of hazelnut leaf contain 3-, 4-, and 5-caffeoylquinic acids, caffeoyltartaric acid, *p*-coumaroyltartaric acid, myricetin 3-rhamnoside, quercetin 3-rhamnoside, kaempferol 3-rhamnoside, *p*-coumaric acid, and myricetin and quercetin derivatives. Most of these bioactive compounds have antibacterial and antioxidant action [10,11]. Furthermore, these components have several hydroxyl and carboxylic groups in their structure which those functional groups can easily reduce metal ions and convert those into their elements, and finally form metal NPs [12]. Leaves of *C. avellana*, an agro-waste, are found in abundance in Iran, and as already mentioned the *C. avellana* leaf extract has reducing activity which can reduce silver ions and convert them into AgNPs, which have a strong antibiotic effect on a wide range of antibiotic resistant microorganisms. However, this process needs a long time at room temperature, due to the low concentration of bioreducing agents in the extract. Microwave heating can effectively increase the collision rate of the formed silver elements and seeds, and decrease the synthesis time [2,3]. Therefore, this study focused on (1) preparation of the aqueous extract of hazelnut leaf and determination of its main functional groups; (2) synthesis of AgNPs and optimization of the amounts of hazelnut leaf extract and silver salt solution to fabricate AgNPs with minimum particle size and maximum concentration; and (3) assessment of their antifungal action against *Colletotrichum coccodes* and *Penicillium digitatum*, which cause tomato and orange spoilage.

2 Materials and method

2.1 Materials

C. avellana leaves were picked from the hazelnut trees in Tabriz, Iran. $AgNO_3$, a silver salt, was bought from Dr Mojallali (Dr Mojallali Chemical Complex Co., Tehran, Iran). Colloidal standard AgNPs solution, with particle size and concentration of 10 nm and 1,000 ppm, respectively, was obtained from Tecnan-Nanomat (Spain). *C. coccodes* and *P. digitatum* were isolated from spoiled tomato and orange. Potato dextrose agar (PDA) was purchased from Oxoid Ltd (Hampshire, United Kingdom). Deionized double distilled water was used to prepare all aqueous solutions.

2.2 Preparation of *C. avellana* leaf extract and synthesis of AgNPs

The *C. avellana* leaves were washed, shade dried, and powdered using domestic miller (MX-GX1521, Panasonic, Tokyo, Japan). Around 1 g of the prepared powder was added into 100 mL of boiling distilled water for 5 min and after that it was cooled to room temperature and filtered using Whatman Grade 1 filter paper. The provided *C. avellana* leaf extract was stored in the refrigerator (at 4°C) throughout the experiment.

According to the literature studies, 1 mM silver nitrate solution (colorless) was prepared by dissolving 0.017 g of $AgNO_3$ in 100 mL deionized double distilled water [1–3]. Different amount of $AgNO_3$ solution (15–25 mL) was mixed with different amount of *C. avellana* leaf extract (0.1–0.9 mL) and the mixture solutions were placed in the microwave oven (MG-2312W, LG Co., South Korea), at a constant power of 800 W and microwave heating time of 180 s.

2.3 Physico-chemical assay

The main functional groups related to the bioactive compounds of *C. avellana* leaf extract, which play the main role in the reduction and stabilization of the AgNPs, were determined using Fourier transform infrared (FT-IR) spectroscopy (Bruker Tensor 27 FT-IR spectrometer, Germany) with KBr pellets in the 4,000–400 cm^{-1} region. The formation of the AgNPs using *C. avellana* leaf extract was confirmed by a Jenway ultraviolet-visible (UV-Vis) spectrophotometer 6705 (United Kingdom). AgNPs due to their surface plasmon resonance (SPR) have broad emission peaks (λ_{max}) in the wavelength ranging from 380 to 450 nm. This is responsible for the striking yellow–brown color of the synthesized AgNPs in various media [8]. UV-Vis spectroscopy measurements can also be used to evaluate the concentration of the AgNPs solution by establishing a standard curve using several serial dilute solutions of AgNPs (10–1,000 ppm). In fact, the absorbance unit at the place of broad emission peak (λ_{max}) can be correlated to the concentration of the synthesized AgNPs in the colloidal solution. Mean particle size, polydispersity index (PDI), zeta potential values, and particle size distribution (PSD) of the fabricated AgNPs

were measured using a dynamic light scattering (DLS) particle size analyzer (Nanotrac Wave, Microtrac, United States) at 25°C. For morphology analysis of the fabricated AgNPs, including size and shape, transmission electron microscopy (TEM) was used. For this reason, the aliquot of the formed AgNPs solution was put on a carbon-coated copper grid and the assay was completed using the instrument (TEM, CM120, Philips, Amsterdam, Netherlands) with an acceleration voltage of 120 kV.

2.4 Antifungal assay

The inhibition in the radial mycelia growth of *C. coccodes* and *P. digitatum* on the poured and dried plates with PDA incorporated with AgNPs was measured for 7 days at a temperature of 26 ± 2°C. In this method, an agar disk (5 mm in diameter) from a pure culture of the fungus was placed in the center of the PDA plates (90 mm in diameter) as control sample and those amended with AgNPs and leaf extract, and the plates were then incubated. Daily radial measurements of mycelia growth were taken until the fungus reached the edge of the control plates. Antifungal activity of the synthesized AgNPs was expressed as inhibition of the fungal hyphae growth (mm).

2.5 Optimization of the AgNPs synthesis conditions

As can be clearly observed in Table 1, experiments were randomly designed using central composite design (CCD), and response surface methodology (RSM) was used to evaluate the effects of the selected synthesized parameter on λ_{max} (nm) and concentration (ppm) of the fabricated AgNPs. According to our previous studies, two AgNPs synthesis parameters, namely *C. avellana* leaf extract (0.10–0.90 mL, X_1) and 1 mM silver salt solution (15–25 mL, X_2), were selected to be optimized for the synthesis of AgNPs with minimum particle size, as manifested in place of λ_{max}, and maximum concentration, based on the absorbance unit of λ_{max} and standard equation [1–3]. As clearly observed in Table 1, 13 experimental treatments were assigned with five different levels for each synthesis parameter [13]. In order to model the λ_{max} (nm) and concentration (ppm) of the synthesized AgNPs to the synthesis parameters, a second order polynomial equation was selected [14]. The suitability of the generated models was studied accounting for the coefficient of determination (R^2) [15,16]. The R^2 and the p-value of the lack of fit were used to assess the fitness and accuracy of the generated models according to the obtained experimental values. The one-way analysis of variance was also used to provide the significance determinations of the resulted models in term of p-value (<0.05) [17]. In order to obtain the optimum synthesis conditions with the desired response variables, numerical multiple response and graphical optimizations were used [17]. Three additional approval tests were performed at obtained optimum synthesis conditions to verify the validity of the statistical experimental method [18]. The Minitab software (v.16 statistical package, Minitab Inc., PA, United States) was used to design experiments, statistical analysis, and optimization.

Table 1: Experimental runs according to the CCD and response variables for AgNPs synthesis

Sample no.	Amount of leaf extract (mL)	Amount of silver salt (mL)	λ_{max} (nm)	Concentration of AgNPs (ppm)
1	0.10	20.00	414	71
2	0.78	16.46	424	133
3	0.78	23.53	419	111
4	0.50	15.00	424	99
5	0.50	25.00	413	67
6	0.50	20.00	427	76
7	0.50	20.00	421	72
8	0.22	23.53	412	72
9	0.50	20.00	423	67
10	0.90	20.00	421	101
11	0.22	16.46	418	73
12	0.50	20.00	427	76
13	0.50	20.00	421	63

3 Results and discussion

3.1 Fabrication of AgNPs

Colloidal mixture solutions containing synthesized AgNPs indicated broad emission peaks (λ_{max}) ranging from 380 to 450 nm due to their SPR [10]. As clearly observed in Table 1, the λ_{max} of the synthesized AgNPs was changed from 412 to 427 nm which those values were in the mentioned λ_{max} range for the AgNPs. This indicated that the *C. avellana* leaf extract successfully reduced silver ions and formed AgNPs.

Figure 1 shows the FT-IR spectrum of *C. avellana* leaf extract which contained four main peaks. The absorption

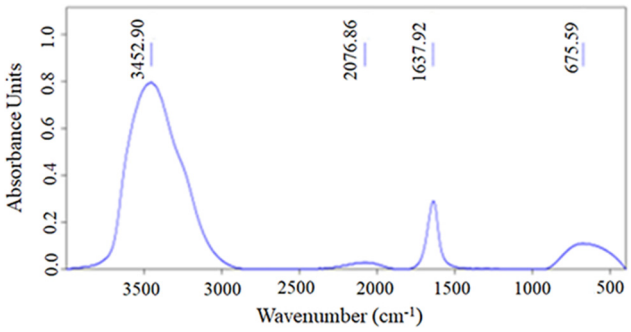

Figure 1: The FT-IR spectrum of *C. avellana* leaf extract.

band at 3452.90 cm^{-1} was related to the stretching vibrations of the hydroxyl group which is the main functional group in the chemical structure of alcohols and phenolic compounds and responsible for reducing silver ions into silver atoms [2]. The observed band at 2076.86 cm^{-1} was corresponded to the C═N and C═C stretching, and the band centered at 1637.92 cm^{-1} was related to O–H bending in carboxylic acid (COOH) groups. These functional groups were in the chemical structure of 4- and 5-caffeoylquinic acids, caffeoyltartaric acid, *p*-Coumaroyltartaric acid, and *p*-Coumaric acid, the main existed carboxylic acids, which those were found in the hazelnut leaf [10,11]. The peak centered at 675.59 cm^{-1} was related to the C–C ring deformation that was observed in the chemical structure of the main bioactive compounds present in the prepared hazelnut leaf extract [9]. FT-IR analysis indicated that the prepared extract had several reducing bioactive compounds with hydroxyl and carboxylic acid groups in their structures, which could effectively reduce silver ions and convert them into AgNPs [8,19].

3.2 Model generation

According to the obtained experimental data for the response variables (Table 1), final models were generated for λ_{max} (Y_1) and concentration (Y_2) of the synthesized AgNPs (Eqs. 1 and 2):

$$Y_1 = 290 - 39.5X_1 - 20.7X_2 + 238.3X_1^2 + 0.5X_2^2 - 5.4X_1X_2, \quad (R^2 = 96.03\%) \tag{1}$$

$$Y_2 = 347.5 + 43.7X_1 + 7.2X_2 - 38.5X_1^2 - 0.2X_2^2 + 0.2X_1X_2, \quad (R^2 = 86.19\%) \tag{2}$$

where X_i, X_i^2, and X_iX_j correspond to the linear, quadratic, and interaction effects of the amount of leaf extract (X_1) and amount of silver salt (X_2). Statistical analysis indicated that the linear effect of the amount of silver salt and the quadratic effects of both the selected synthesis parameters had significant ($p < 0.05$) effects on λ_{max} and concentration of the synthesized AgNPs. The high values for R^2 of λ_{max} and concentration of the synthesized AgNPs confirmed the suitability of the resulting models. Barabadi et al. also synthesized AgNPs induced by *Penicillium citrinum* and reported that the obtained R^2 value of 0.889 for the fitted model indicated a good correlation between the observed and predicted response values [20].

3.3 Optimization of the AgNPs synthesis parameters

The aim of the optimization for AgNPs synthesis conditions is fabrication of AgNPs with minimum particle size, which could be manifested in the small values for λ_{max} and maximum concentration. In order to indicate the optimum area for AgNPs synthesized parameters, graphical optimization using an overlaid contour plot was plotted (Figure 2). The white colored area in Figure 2 indicates the desired amounts of *C. avellana* leaf extract and $AgNO_3$ solution to synthesize AgNPs with small particle size and high concentration. A numerical multiple optimization also indicated that 0.9 mL of *C. avellana* leaf extract and 25 mL of $AgNO_3$ would give the most desirable NPs with the λ_{max} of 412 nm and concentration of 140 ppm.

According to the provided standard curved using several serial dilute solutions of the prepared standard AgNPs colloidal solution, the standard Eq. 3 was obtained as

$$C = 26.75X - 0.54 \tag{3}$$

where C and X are the concentration and the absorbance units at λ_{max}, respectively. As can be clearly observed in

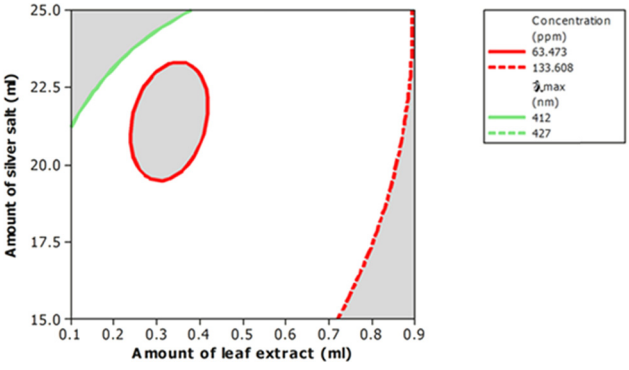

Figure 2: Overlaid contour plot of AgNPs λ_{max} and concentration with acceptable levels as a function of the amount of *C. avellana* leaf extract and amount of $AgNO_3$ solution.

Figure 3, the absorbance unit of the diluted solution (one time) containing synthesized AgNPs, using obtained optimum conditions, was 2.62. The obtained results revealed that using the obtained optimum conditions, AgNPs with a concentration of 140 ppm were synthesized. Ahmadi et al. also studied green synthesized AgNPs using *Aloe vera* leaf extract and reported that the fabricated AgNPs using obtained optimum amount of 1 mM silver salt solution (9 mL) and microwave heating time (360 s) had λ_{max} and concentration of 410 and 64 ppm, respectively [2].

3.4 Characteristics of the synthesized AgNPs at obtained optimum conditions

The fabrication of AgNPs was confirmed by the observation of a broad emission peak in the UV-Vis spectra of the colloidal solution containing synthesized AgNPs using obtained optimum conditions (Figure 3a). Furthermore, DLS analysis demonstrated that these NPs had mean particle size, PDI, and zeta potential values of 103.5 nm, 0.209, and −21.8 mV, respectively. The PSDs of the produced AgNPs are shown in Figure 3b. DLS analysis of the synthesized AgNPs using *A. vera* leaf extract and microwave heating by Ahmadi et al. indicated that the fabricated NPs had particle size and zeta potential values of 46 nm and +15.5 mV, respectively [2]. The small particle size of the AgNPs synthesized by Ahmadi et al., compared to the particle size of the fabricated AgNPs in our study, could be explained by the fact that, by increasing the microwave heating time, the collision rate of the formed NPs increased which reduced the particle size of the AgNPs [3]. The morphology of the fabricated AgNPs at optimum synthesis conditions is presented in Figure 4. As can be observed in the TEM image, the spherical shaped AgNPs were well dispersed in the colloidal solution. The spherical shape could be related to their minimum surface energy and their wide dispersion could be correlated to their high thermodynamic stability, because of the high value of the zeta potential [1–3,21].

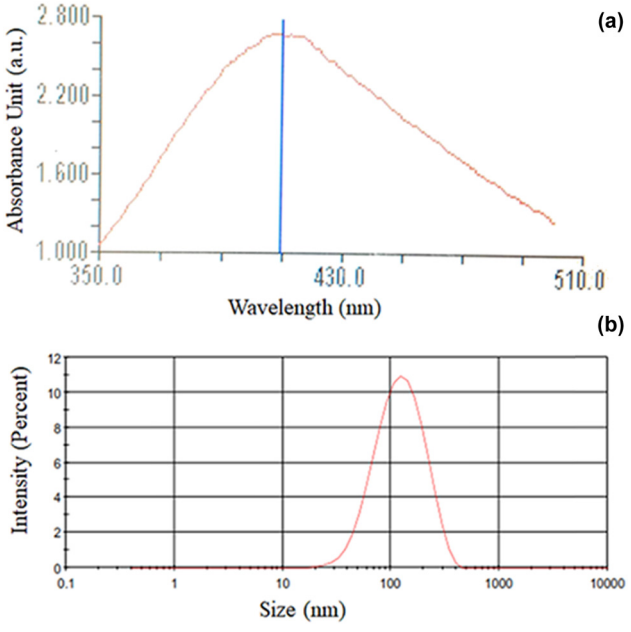

Figure 3: UV-Vis spectra (a) and PSD (b) of the mixture solution containing synthesized AgNPs at optimum synthesis conditions.

3.5 Antifungal activity of the synthesized AgNPs at obtained optimum conditions

The antifungal activity of the synthesized AgNPs using *C. avellana* leaf extract as manifested in the inhibition of mycelia growth of *C. coccodes* and *P. digitatum* is shown in Figure 5. As can be seen in these figures, the synthesized AgNPs using *C. avellana* leaf extract showed high fungicidal activity against both the selected fungi strains. As can be observed in Figure 5a, after 7 days of incubation, the diameters of the mycelia growth of *C. coccodes* in the control plate and the one containing PDA and the synthesized AgNPs were 42 and 33 nm, respectively. On the other hand, the diameters of the mycelia growth of *P. digitatum* in the control plate and the one containing PDA and the synthesized AgNPs were 39 and 16 nm, respectively (Figure 5b). The obtained results revealed that the fabricated AgNPs could effectively inhibit the mycelia growth of *P. digitatum* compared to that of *C. coccodes*.

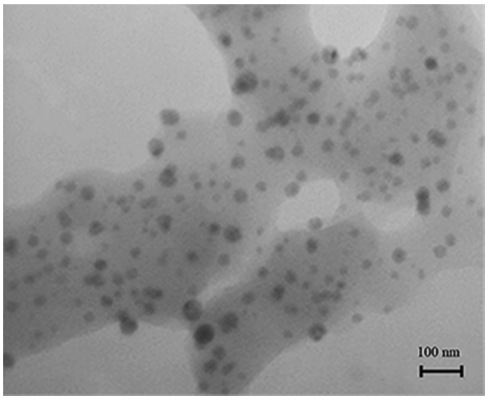

Figure 4: The TEM image of the synthesized AgNPs at obtained optimum synthesis conditions using the *C. avellana* leaf extract.

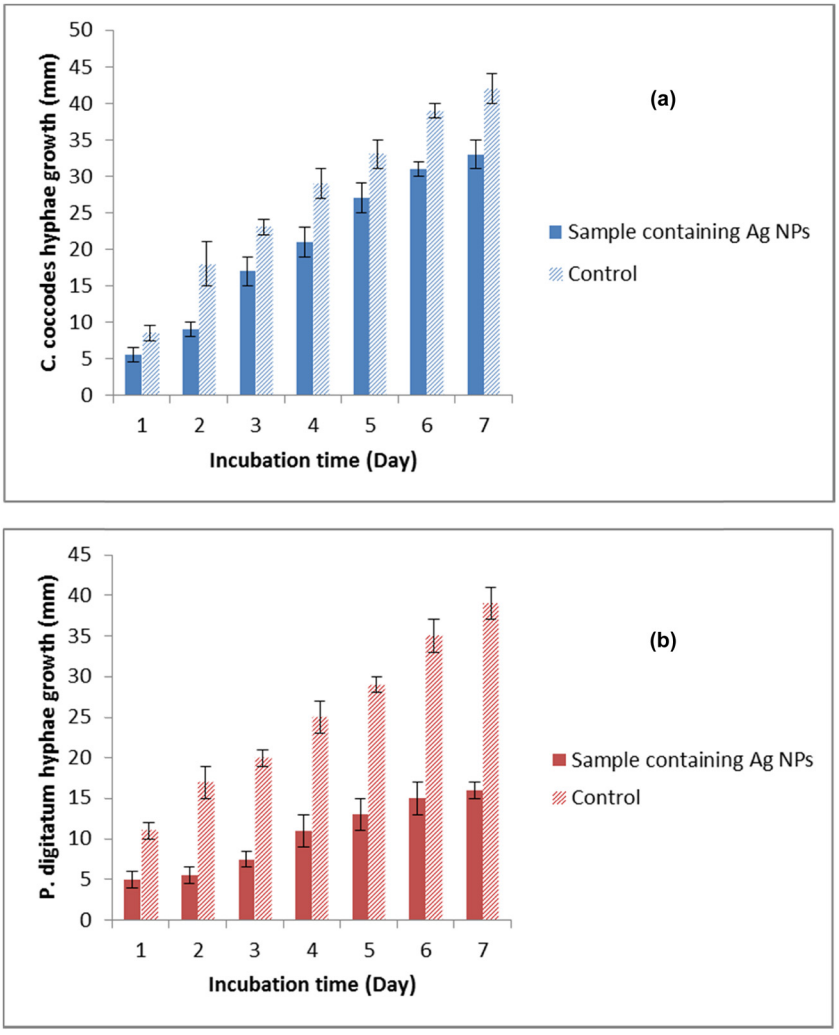

Figure 5: Effect of the synthesized AgNPs on inhibiting the mycelia growth of *C. coccodes* (a) and *P. digitatum* (b). Values are mean of the three replicates.

In fact, the surface of AgNPs can easily form a layer of water and thus many silver ions can be released from AgNPs into the water. On the other hand, the main composition of the fungus cell membrane is phospholipid bilayers and protein molecules having negative electricity which make the whole cell membrane negatively charged. Therefore, the silver ions with positive electricity have the ability to attach to the cell membrane quickly, which alters or damages their structures [22–24]. The obtained results were in line with the finding of Mohammadlou et al. [1]. They found that the synthesized AgNPs using *Pelargonium* leaf extract, effectively inhibited the growth of *Aspergillus flavus* and *Aspergillus terreus*. Jafarizadeh-Malmiri et al. successfully used edible coating based on carboxymethyl cellulose and AgNPs to inhibit the growth of *Colletotrichum musae* on banana fruit and increased its shelf life [23].

4 Conclusion

The hazelnut leaf extract indicated high reduction potential to reduce silver ions and convert them into AgNPs. Microwave heating could also accelerate the fabrication rate of AgNPs to minimize energy consumption. The obtained results indicated the usefulness of RSM for studying the effects of the synthesis conditions on response variables and their optimization to synthesize spherical AgNPs with more desirable physicochemical characteristics and high fungicidal activity. The developed intensified synthesis process to fabricate AgNPs, based on using hazelnut leaf extract, microwave heating, and obtained optimum conditions, can be used to synthesize other novel metal and metal oxide NPs. Furthermore, the provided colloidal solutions containing synthesized AgNPs and hazelnut leaf extract can be utilized in various areas that need antimicrobial agents in their products.

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