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Bioproduction of Silver Nanoparticles by *Myrtus Communis* Leaf Extract and Their Effect on Plant Pathogenic Fungi *in Vitro*

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In this study, *Myrtus communis* was used for the bioproduction of colloidal silver nanoparticles (AgNPs). The resultant AgNPs were characterized by X-ray diffraction (XRD), UV-Visible spectroscopy and field-emission scanning electron microscopy (FESEM). The hump-like peaks were recorded near the wavenumber 422 nm and such peaks originated due to the electronic structures of silver tiny particles and silver nanoparticles. The four intensive peaks of XRD patterns indicated the crystalline nature and the face-centered cubic structure of the AgNPs. The average crystallite size of the AgNPs ranged from 19 nm to 25 nm. The FESEM image illustrates the good dispersion of the AgNPs and the spherical shape of the nanoparticles. The AgNPs were prepared to study antifungal activity against plant pathogenic fungi *Aspergillus niger*, *Rhizopus stolonifer* and *Neurospora crassain vitro*. Results showed that the AgNPs demonstrated high antifungal activity against these fungi with significant differences between treatments when compared with the control (without nanoparticles).

Keywords: Bioproduction; nanoparticles; *Aspergillus*; *Rhizopus*; *Neurospora*; silver particles; antifungal activity.

1. Introduction

Nanoparticles are classified as materials with one or more dimensions between 1 nm and 100 nm in size,[1] and they are considered metallic oxides, metalloids, nonmetals, and nanomaterials. Given their small scale, large surface area, and high reactivity, they are useful as bactericides, fungicides and nano-fertilizers.[2] Plants and microorganisms have been

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used to synthesize nanoparticles *in vivo*, and there is a large body of literature on this topic. Bioproduction methods vary, but organic resources or their extracts are exposed to a metallic salt that biologically reduces the metal to a nanoscale size, which is then collected, distinguished, and made available for use.[3]

Plant disease reduces agricultural production every year, and many strategies can be used to control plant disease, such as use of pesticides. However, in recent years, scientists in the agricultural sector have used silver nanoparticles (AgNPs) in plant disease management.[4,5] When AgNPs are used, sclerotium-forming fungi decrease, which is considered the most effective protection mechanism for controlling plant pathogens.[6]

Researchers are attempting to determine how to make AgNPs in the safest way possible. One of the most popular and quickest ways to prepare nanoparticles is to use extracts from plant leaves, seeds, and other sources. Plant ingredients, such as *Aloe vera*, have been used by a number of scientists in the preparation of nanoparticles.[7] According to Kantha and Arunachalam,[8] preparing AgNPs and gold nanoparticles from plant leaf water extracts is potentially environmentally friendly, and the water extraction method can be used to produce them quickly. Different quantities of extract can be used to change the size of nanoparticles. Plants and plant extracts take precedence over physico-chemical methods.[9,10] Plant-mediated nanoparticle processing is favored over other known methods because it is safe for human use, low in cost, and environmentally friendly.[11] According to Mubarak Ali I-Nan LinK,[12] by increasing the molar concentration of gold precursor, the size of nanoparticles increased up to the size of particles.

This study aimed to prepare AgNPs using *Myrtus communis* plant extract, to characterize and analyze AgTPs and AgNPs prepared at different molar concentrations and study their effect on fungi *in vitro*.

2. Materials and Methods

2.1. *M. communis extract preparation*

Leaves of *M. communis* were collected from the University of Anbar, washed with distilled water, and dried for 24 h in an incubator at 45°C. About 5 g of dry leaves was mixed with 500 mL of distilled water in a flask, which was centrifuged at 3000 rpm. The solution was filtered to obtain extracts with a concentration of 100%.[13]

Myrtus.communis extract

2.2. *Bioproduction of AgNPs*

M. communis extract was used to convert $AgNO_3$ into AgNPs for AgNP bioproduction. About 1 mL of $AgNO_3$ (0.4 mM, 0.6 mM and 0.8 mM) was applied to 45 mL of *M. communis* extract in a 250 mL flask. The flask was placed on a magnetic stirrer at 80°C for 60 min.[14,15]

2.3. *UV-Vis spectrophotometry*

A UV-Visible spectrophotometer was used to track the bioproduction of AgNPs on a regular basis. To analyze this sample and research the formation of AgNPs at 350–650 nm, 0.1 mL of the sample was diluted in 2 mL of deionized water.

2.4. *Diagnosis of AgNPs by field-emission scanning electron microscopy (FESEM)*

The films on the FESEM grids were permitted to stand for 2 min after being dipped in an aqueous solution of AgNPs. The excess solution was extracted with blotting paper, and the grid was allowed to dry until measurement.[16]

2.5. *Antifungal activity of the prepared AgNPs in vitro*

The *in vitro* assay involved one type of growth medium (PDA) treated with diverse concentrations (0 mM, 0.4 mM, 0.6 mM and 0.8 mM) of AgNPs. About 2 mL of AgNPs of various concentrations was poured into a Petri dish with PDA. Growth media with AgNPs were inoculated with fungi at the center of each Petri dish and then incubated at 30°C for 2 weeks.[17]

2.6. *Calculation of inhibition rate (%)*

The radial growth of fungi was measured after they were incubated on various growth media containing AgNPs. When mycelial growth in the control Petri dish reached the end of the plate, the inhibition ratio was calculated. The inhibition rate (%) was calculated using the following formula[17]:

$$\text{Inhibition rate}(\%) = R - r/R,$$

R = radial growth of fungi mycelia on the control Petri dish.

r = radial growth of fungi mycelia on the Petri dish treated with AgNPs.

3. Results

AgNPs were synthesized using the natural materials of plants. They were first identified by the color change in the reaction to a ruby red color. UV-Vis absorption spectra were used to study the intensity of absorbed field of AgTPs and AgNPs synthesized for different molar concentrations. Figure 1 shows the absorption peaks of the UV-Vis spectra of aqueous media of AgNPs as a function of silver nitrate salt's concentration.[18] In the UV-Vis spectra of AgNPs prepared at 0.6 mM, we observed the absorption peak at 422 nm. This peak indicated the formation of the spherical shape of AgNPs. The shift in the wavelength (from 422 nm to 416 nm) of the AgNP spectra indicated an increase in the concentration of elongated and deformed metal (silver) atoms.[19] The higher the metal ion concentration, the higher the particle size of the AgNPs. The sharp peak around 422 ± 2 nm indicated AgNP formation.[20] Meanwhile, the enhancement of the intensity might be related to the enlargement of the formed nanoparticles when the number of silver ions decreased in the aqueous solution, thereby increasing the absorbance intensity of the AgNPs. This result was in an agreement with the findings reported by Edreese Alsharaeh *et al.*,[21] who utilized lemon juice under microwave irradiation and UV light irradiation to synthesize AgNPs. The reported results revealed that the maximum absorption peak of the spherical AgNPs was located between 420 nm and 450 nm. Pandian Bothi Raja *et al.*[20] employed a rapid green method to synthesize AgNPs by using a variety of tannin sources such as mangrove (MG), chestnut (CN) and quebracho (QB) as reducing agents. They revealed that the absorbance peak areas at 430–450 nm increased as the reaction time increased. Jae Yong Song *et al.*[18] reported the use of five plant leaf extracts (e.g., pine and persimmon). The peaks related to all samples show the high intensity (in arbitrary unit), and this validates the electronic structures of AgTPs and AgNPs. In Fig. 1, each peak's trend is different. In Fig. 1, the hump-like peaks are related to the absorbed high-field intensity. These attribute the electronic structures of silver tiny particles and silver nanoparticles. At different molar concentrations, the electronic structures of (AgTPs and) AgNPs also become different regardless of that a silver atom has fixed number of electrons.[19,21,22]

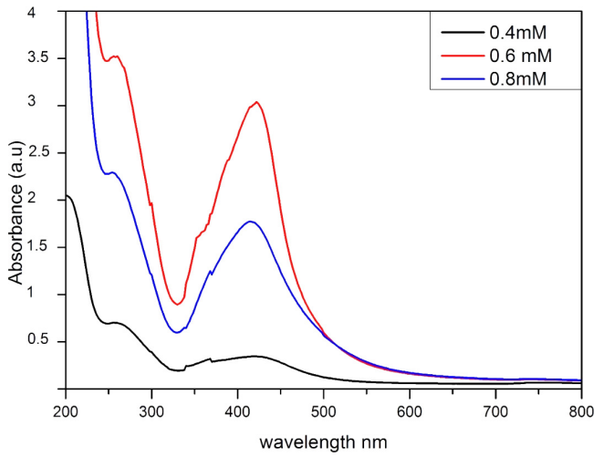

Fig. 1. Visual and ultraviolet spectra of AgNPs prepared using *M. communis* leaf extract as a function of the molar concentration of $AgNO_3$.

X-ray reflection (XRF) patterns of the dried AgNPs (see Fig. 2) showed the partially

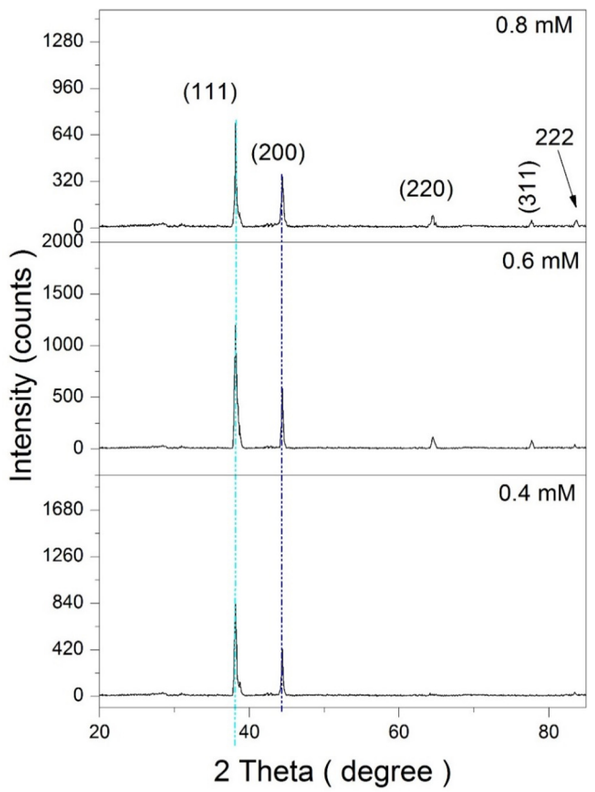

Fig. 2. The results of the XRF examination of prepared using *M. communis* leaf extract as a function of the molar concentration of $AgNO_3$.

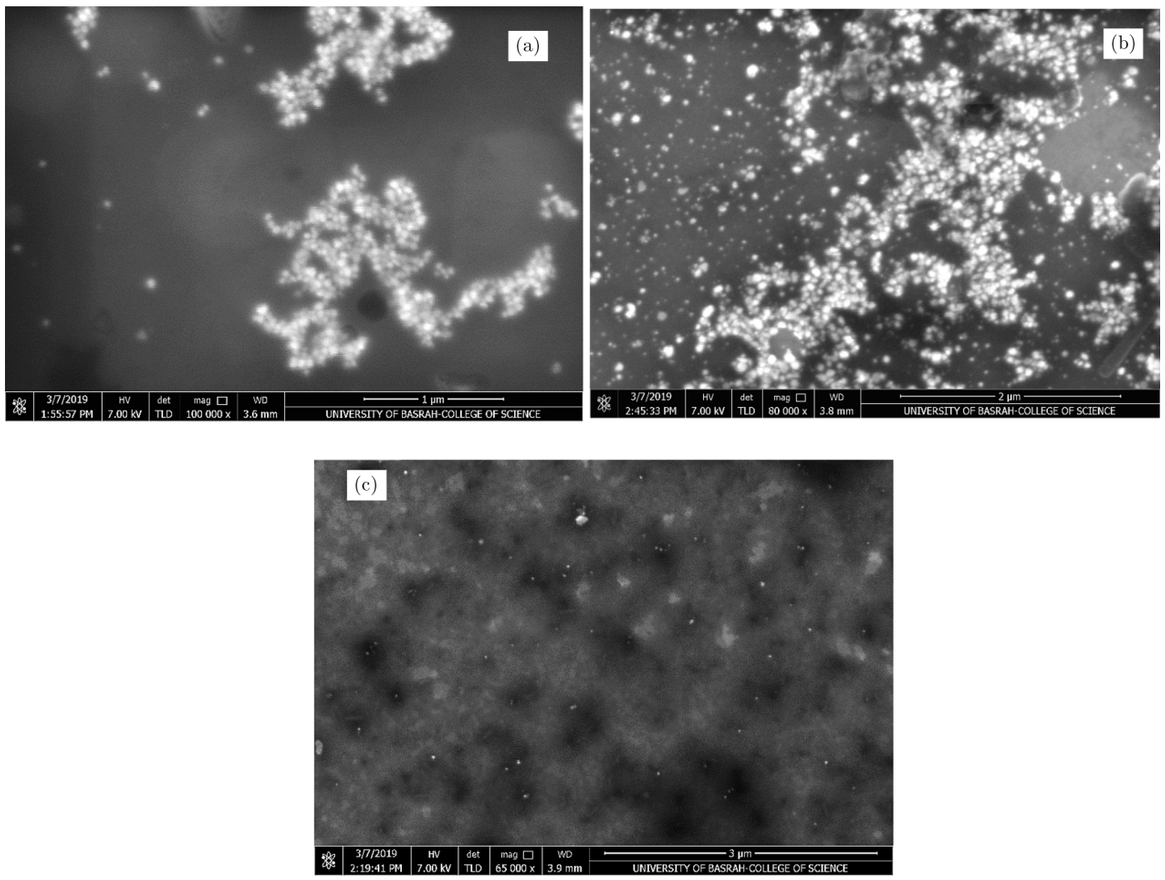

Fig. 3. FESEM image of AgNPs prepared using *M. communis* leaf extract as a function of the molar concentration of $AgNO_3$: (a) 0.4 mM, (b) 0.6 mM and (c) 0.6 mM.

crystalline-partially non-crystalline (amorphous) structure of AgNPs synthesized using *M. communis* leaf extract as a function of the metal atom concentration. Consequently, the peaks of 2θ at 38.45°, 44.39°, 64.57° and 77.54° corresponded to the different electronic rings of silver atoms belonging to the top-layered surface of AgTPs and AgNPs.[23] No extra peaks belonging to other crystalline phases were detected, indicating the pure synthesis of AgTPs and AgNPs without impurities. The XRF peaks of our synthesized AgNPs agreed with the gold standard peaks (JCPDS pattern; file no. 04-0784). The peak at 38.18° of the (1 1 1) plane exhibited stronger intensity than the peaks at 44.39°, 64.57° and 77.54°. The number of peaks and their intensities are affected by the change in metal ion concentration. At a low concentration (0.4 mM), the intensity of the four detected peaks was higher than that at high concentrations (0.6 mM and 0.8 mM). The crystalline size distribution was between 19 nm and 25 nm with an average crystallite size of 22 nm. The higher the metal ion concentration is, the bigger is the size of particle. For this, please refer to the published study, Ref. 23.

FESEM was used to investigate the morphology of the synthesized AgNPs. As shown in Fig. 3, the AgNPs had a spherical shape and a small particle size for samples prepared with 0.6 mM compared with other samples. This behavior was in agreement with the results of XRD.

3.1. *Antifungal activity of the prepared AgNPs in vitro*

The results of this study showed the effect of AgNPs on the growth diameter (Table 1) and inhibition ratio (Table 2) of plant pathogenic fungi *Aspergillus niger*, *Rhizopus stolonifer* and *Neurospora crassa*. Significant differences were found among all treatments. The concentration of 0.8 mM gave the best

Table 1. Effect of AgNPs on the growth diameter of plant pathogenic fungi.

Growth diameter (cm)			
Concentration ($AgNo_3$)	*Aspergillus niger*	*Rhizopus stolonifer*	*Neurospora crassa*
0.4 mM	7.30 + 0.115	7.90 + 0.115	7.53 + 0.088
0.6 mM	4.80 + 0.152	5.43 + 0.185	6.33 + 0.088
0.8 mM	3.16 + 0.176	3.33 + 0.088	3.80 + 0.057
Control	8.73 + 0.145	8.83 + 0.088	8.90 + 0.057

$P = 0.000$.

Table 2. Effect of AgNPs on the inhibition ratio of plant pathogenic fungi.

Inhibition ratio (%)			
Concentration ($AgNo_3$)	*Aspergillus niger*	*Rhizopus stolonifer*	*Neurospora crassa*
0.4 mM	16.38	10.53	15.39
0.6 mM	45.01	38.50	28.87
0.8 mM	63.80	62.28	57.30
Control	0	0	0

effect on the growth diameters of *A. niger*, *R. stolonifer* and *N. crassa* (3.16 cm, 3.33 cm and 3.80 cm, respectively) compared with the control (8.73 cm, 8.83 cm and 8.90 cm, respectively; Table 1), with inhibition ratios reaching 63.80%, 62.28% and 57.30%, respectively (Table 2). The concentration of 0.6 mM inhibited the growth diameters of *A. niger*, *R. stolonifer*, and *N. crassa* to 4.80 cm, 5.43 cm and 6.33 cm, respectively, and the inhibition ratios reached 45.01%, 38.50% and 28.87%, respectively (control = 0%).

The poor effects of AgNPs on the growth diameter and inhibition ratio of fungi at 0.4 mM yielded diameters of 7.30 cm, 7.90 cm and 7.53 cm (Table 1) and inhibition ratios of 16.38%, 10.53% and 15.39% (control = 0%; Table 2) for *A. niger*, *R. stolonifer* and *N. crassa*, respectively.

4. Conclusion

The use of materials, including AgNPs, within the nanoscale in medical applications has become commonplace due to their unique properties. An environmentally friendly method was used to prepare AgNPs from silver nitrate, as this method is considered one of the easiest, economical, and safest methods in the field. XRF patterns of the dried AgNPs showed the partially crystalline-partially non-crystalline (amorphous) structure of AgNPs. FESEM shows that the AgNPs had a spherical shape and a small particle size. This study showed the importance of nanoparticles in reducing fungal plant diseases; it showed that the concentration of 0.8 mM was superior to the rest of the concentrations in the effect on the studied fungi: *Aspergillus niger*, *Rhizopus stolonifer* and *Neurospora crassa*, then the concentration of 0.6 mM came second in terms of the effect on fungi, and then the concentration 0.4 mM as compared to the control standard.

References

1. B. S. Sekhon, *Nanotechnol. Sci. Appl.* **7**, 31 (2014).
2. W. Elmer and J. C. White, *Annu. Rev. Phytopathol.* **56**, 111 (2018).
3. A. K. Mittal, Y. Chisti and U. C. Banerjee, *Biotechnol. Adv.* **31**, 346 (2013).
4. A. K. Mittal, Y. Chisti and U. C. Banerjee, *Biotechnol. Adv.* **31**(2), 346 (2013).
5. J. S. Min, K. S. Kim, S. W. Kim, J. H. Jung, K. Lamsal, S. B. Kim and Y. S. Lee, *Plant Pathol. J.* **25**, 376 (2009).
6. M. Sastry, A. Ahmad, M. I. Khan and R. Kumar, *Curr. Sci.* 162 (2003).
7. S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, *Biotechnol. Prog.* **22**, 577 (2006).
8. K. D. Arunachalam, S. K. Annamalai and S. Hari, *Int. J. Nanomed.* **8**, 1307 (2013).

9. P. Sivakumar, C. Nethradevi and S. Renganathan, *Asian J. Pharm. Clin. Res.* **5**, 97 (2012).
10. P. Kouvaris, A. Delimitis, V. Zaspalis, D. Papadopoulos, S. A. Tsipas and N. Michailidis, *Mater. Lett.* **76**, 18 (2012).
11. V. Kumar and S. K. Yadav, *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean Technol.* **84**, 151 (2009).
12. M. Ali and I.-N. Lin, *Adv. Nat. Sci. Nanosci. Nanotechnol.* **11**, 015006 (2020).
13. P. G. Ndegwa, Assessment of Factors Influencing Food Security in Wenje Division, *Tana River County-Kenya (Doctoral dissertation, Pwani University)* (2015).
14. T. A. Salih, K. T. Hassan, S. R. Majeed, I. J. Ibraheem, O. M. Hassan and A. S. Obaid, *Biotechnol. Rep.* **28**, e00545 (2020).
15. I. J. Ibraheem, K. T. Hassan, H. H. Ali and A. S. Obaid (2020). *Nano Biomed. Eng.* **12**, 331.
16. A. Abdel-Azeem, A. A. Nada, A. O'donovan, V. K. Thakur and A. Elkelish, *J. Renew. Mater.* **8**, 171 (2020).
17. S. W. Kim, J. H. Jung, K. Lamsal, Y. S. Kim, J. S. Min and Y. S. Lee, *Mycobiology* **40**, 53 (2012).
18. J. Y. Song and B. S. Kim, *Bioprocess Biosyst. Eng.* **32**, 79 (2009).
19. M. Ali and I.-N. Lin, *Adv. Nat. Sci. Nanosci. Nanotechnol.* **10**, 025015 (2019).
20. E. Alsharaeh, S. Alazzam, F. Ahmed, N. Arshi, M. Al-Hindawi and G. K. Sing, *Acta Metal. Sinica (English Letters)* **30**, 45 (2017).
21. M. Ali and I.-N. Lin. Effects of the Electronic Structure, Phase Transition and Localized Dynamics of Atoms in the Formation of Tiny Particles of Gold (2020), https://arxiv.org/abs/1604.07144v20.
22. M. Ali, I.-N. Lin and C.-J. Yeh. *Nano* **13**, 1850073 (2018).
23. Ali, Mubarak (2021). Qualitative Analyses of Thin Film-Based Materials Validating New Structures of Atoms. 10.13140/RG.2.2.27720.65287.