

RESEARCH PAPER

Role of Rosmarinus Officinalis L Extract as a Bio-Reducing Agent for the Synthesis of Nanomaterials and its Use as a Fungicide

Nidhal Abdul Hussein Al-Bdairi ^{1*}, Mohammed Fakhri AL-Khafaji ²

¹ Department of Biology, Faculty of Science, Kufa University, Najaf, Iraq

² The Babylon Governorate Education Directorate, Babylon, Iraq

ARTICLE INFO

Article History:

Received 10 February 2026

Accepted 14 May 2026

Published 01 July 2026

Keywords:

Biosynthesis of Nanoparticles

Fungicide

Nanotechnology

R. officinalis

ABSTRACT

The manufacture of nanoparticles using plant extracts is an important and useful biological process compared to other manufacturing methods. The reason for this is that the biological or green nanotechnology method where there is no problem in maintaining and preserving plant cell cultures, so there is no fear of mutation in the preparation medium, as is the case with bacterial or fungal cultures. It is also easy to prepare and manufacture, usually in a few quick steps, and is inexpensive. The aim of this research was to find a safer, more environmentally friendly, inexpensive, and harmless method of synthesizing silver nanoparticles from *Rosmarinus officinalis* extract and using them as a fungicide. The nanoparticles (silver nanoparticles) were Biosynthesized by using the aqueous extract of the rosemary plant and using it as a biological agent to reduce silver nitrate to nano-sized silver ions, by distilling the plant extract on the silver nitrate solution and observing the color change process, which indicates the formation of silver nanoparticles. Scientific tests confirming the green manufacturing process were conducted. Silver nanoparticles were then used as a fungicide in the laboratory for fungi that produce certain mycotoxins. Silver nanoparticles can be synthesized from *R. officinalis* leaf extract. The silver nanoparticles appeared to have a semi-pyramidal shape with a size of 63.65 nm when examined by Scanning Electron Microscopy, UV/Vis spectroscopy showed a peak at a wavelength of 345 nm, while X-ray diffraction showed four distinct diffraction peaks that matched the standard with which it was compared. The Atomic Force Microscopy examination showed that the size of the surface topography was 29.65 nanometers. Test results showed the effectiveness of three concentrations of silver (Ag) nanoparticles on certain pathogenic fungi that produce mycotoxins. The results of the experiment demonstrated the effectiveness of these nanoparticles. was very high in inhibiting the studied fungal species on PDA medium. It was observed that the percentage of inhibition increased with increasing concentrations, as the inhibition results at the concentration (ppm /L 100) of silver nanoparticles showed 100% complete inhibition with the two species *Aspergillus niger* and *Fusarium solani*, while 73.3% inhibition was observed for the same concentration with *F. verticillioides*. The study confirmed that the biosynthesis of nanoparticles through the use of plant extracts is a safe, fast, inexpensive and effective method. The study showed that nanoparticles prepared from rosemary extracts can be used as an antifungal agent against mycotoxins in the laboratory, with an inhibition rate of 100%.

How to cite this article

Al-Bdairi N., AL-Khafaji M. Role of Rosmarinus Officinalis L Extract as a Bio-Reducing Agent for the Synthesis of Nanomaterials and its Use as a Fungicide. J Nanostruct, 2026; 16(3):3181-3189. DOI: 10.22052/JNS.2026.03.014

* Corresponding Author Email: nidhal.albdairi@uokufa.edu.iq



INTRODUCTION

Plants are an important and primary source of food for most living organisms. Since ancient times, humans have relied on various plant species as a source of sustenance. The existence of humans depends primarily on the existence of plants, which are not only used as a source of food but are also the main source of oxygen in the atmosphere. Plants also play a major role in medicine and pain relief. [1], and since ancient times, plants are natural antioxidants that are used in traditional medicine to treat many diseases [2,3]. Rosemary is one of the most important plants used in ancient and modern times, belonging to the Lamiaceae. In the field of medicine and wound treatment, plant extracts from rosemary have been used to treat many diseases and wounds, as well as to combat the effects of aging (senescence). Extracts from this plant are rich in flavonoids and phenols, which play a crucial and important role in eliminating the harmful effects of free radicals [4].

On the other hand, nanotechnology has attracted a lot of attention and has entered many aspects including medicine, agriculture, biomedicine, environment, engineering, and others [5]. The use of extracts as intermediates in the preparation of nanomaterials is more important than other biological methods (methods using fungi or bacteria) because there is no difficulty in preserving the plant extract, easy handling, no contamination, and no mutations. [6]. Methods that use natural materials or what is known as the biological method that uses materials available in nature, such as plants, algae, or microorganisms such as bacteria and fungi, as this method is preferred in the preparation of nano-sized materials over other physical or chemical methods, also in terms of cost, as it is low cost, fast to prepare, and has no by-products that can be dangerous and toxic to humans or the environment [7]. Plants have a high reservoir of secondary metabolites that can act as reducing agents in the preparation of nanoscale materials [8], and Qasimet *al.* [9] were able to synthesize silver nanoparticles with a size of 36 nm from clove extract and had antibacterial activity. Several researches revealed the use of nanoscale materials to inhibit and kill pathogenic organisms, including bacteria and fungi, enabling researchers to use them as antifungal substances or other pathogenic organisms. [10]. This research aimed to use rosemary extract to synthesize nanosilver and

use it as an antifungal agent.

MATERIAL AND METHODS

Preparation of hot aqueous extract of rosemary leaves

The hot aqueous extract was prepared in the same way as the cold alcoholic extract, replacing the ethanolic alcohol with boiling water, placing the vial in a shaking incubator for one hour at laboratory temperature, filtering with filter paper and placing the filtrate directly without drying in an opaque tube for use in the preparation of nano-sized silver ions [10].

Preparation solutions

In order to biosynthesize Ag- nanoparticles from rosemary extract, a AgNO_3 solution was prepared at a 2 mM (0.340 mg) of AgNO_3 in 1 L of deionized water under special circumstances (dark) with the glass beaker covered with commercial aluminum and the solution was kept in an opaque manner until use [11].

Synthesis of silver nanoparticles from rosemary extract

Silver nanoparticles were synthesized at Al-Amin Center for Research and Advanced Biotechnologies in Najaf, as the synthesis was done by Take 10 ml of the previously prepared plant extract solution and add it dropwise to 90 ml of the previously prepared AgNO_3 solution with placing on a magnetic stirrer for 30 minutes at 35 °C, after which the color change was observed [12].

Tests that confirm nanopreparation

A UV/VIS spectrophotometer was used in the wavelength) 200 to 750 nm(, readings were taken at approximately 28 °C, and the results were used to construct curves for silver nitrate solution and rosemary extract [13].

In order to complete the rest of the assays supporting the formation of silver nanoparticles, the silver nitrate solution and rosemary extract were centrifuged at 15,000 rpm for 15 minutes, the top layer (liquid) was discarded, and the precipitate was washed with deionized water, then centrifuged at 12000 rpm for 10 min, and the process was repeated three times, then the silver nanoparticles were dried in an oven at 40 °C, ground and kept in a dark place, then the nanomaterial was examined by XRD, scanning

electron microscopy and atomic force microscopy [14].

In vitro application of nano-sized silver ions as an antifungal agent

I followed the method described by Sudhakar *et al.*, [15] mixing biosynthesized silver nanoparticles with potato_dextrose_ agar (PDA) culture medium after cooling to approximately 40 °C, at three concentrations (50, 75 and 100) ppm/L and three replicates of each concentration. After solidification of the medium, a 5 mm diameter disk was transferred with a cork borer to the plate medium from the cultures of pathogenic fungi growing on PDA medium at 5 days of age. Two control groups were used, the first without adding any substance to the culture medium, and the second by adding the fungicide Tachigaren 30 L (active ingredient Himexazol 30%) at a concentration of 2 ml per 1 liter of water to the medium, then incubated at a (27±2°C) for 7 days.

Statistical analysis

The laboratory experiments were carried out according to the Complete_Randomized_Design with three replicates and the means were compared according to the (LSD) test under the

significance (0.05).

RESULTS AND DISCUSSION

A study to evaluate the bioefficacy of rosemary leaf extract in the preparation of nano-sized silver ion

This study aimed to investigate the possibility of using the hot aqueous extract of *R.officinalis*. The color began to change with the addition of drops of the extract to the silver nitrate solution (2 molar) with continuous shaking and the color change continued gradually over time until the gray color, then finally settled on the brown color after 24, 48 and 72 hours, which indicates the reduction of silver -nitrate to nano-sized silver ion as shown in Fig. 1.

In order to confirm the formation of silver nanoparticles, the following tests were performed:

UV/Vis Spectrophotometer with an absorption peak at 345 nm wavelength as shown in Fig. 2. X-ray diffraction (XRD) spectroscopy Fig. 3 showed that there were four 2θ peaks corresponding to 111, 200, 220, and 311 from the standard X-ray magnetic resonance spectrogram of silver nanoparticles (JCPDS_ silver: 04-0783).

The surface topography of the silver nanoparticles was studied by atomic force



Fig. 1. Color change of silver nitrate solution (2 molar) After adding the aqueous extract of rosemary leaves.

microscopy. Fig. 4 shows a 2D image of the surface of the silver nanoparticles, Fig. 5 shows a 3D image of the silver nanoparticles, and average size of the nanoparticles at 29.65 nanometers.

The scanning electron microscopy results of the biosynthesized Ag nanoparticles showed that they had a pyramidal shape and sizes (30 - 94 nm), as shown in Figs. 6-9. The average size of the silver nanoparticles examined by scanning electron microscopy with the help of Image J software was calculated to be 63.65 nm.

A study to evaluate the efficacy of AgNPs as a fungicide

The results of testing the efficacy of three

concentrations of biosynthesized AgNPs on some mycotoxin-producing pathogenic fungi, which had an average size of 63. The results revealed the effectiveness of AgNPs in inhibiting the studied fungal species on PDA medium, as it was observed that the percentage of inhibition increased with increasing concentrations, as the results of inhibition at the concentration (ppm / L 100) of silver nanoparticles showed 100% complete inhibition with the two species *F. solani* and *A. niger*, while the percentage of inhibition reached 73.3% for the same concentration with *F. verticillioides*, while the concentration (ppm/L 50) showed varying inhibition rates as the highest inhibition rate reached 78.8% with a growth rate

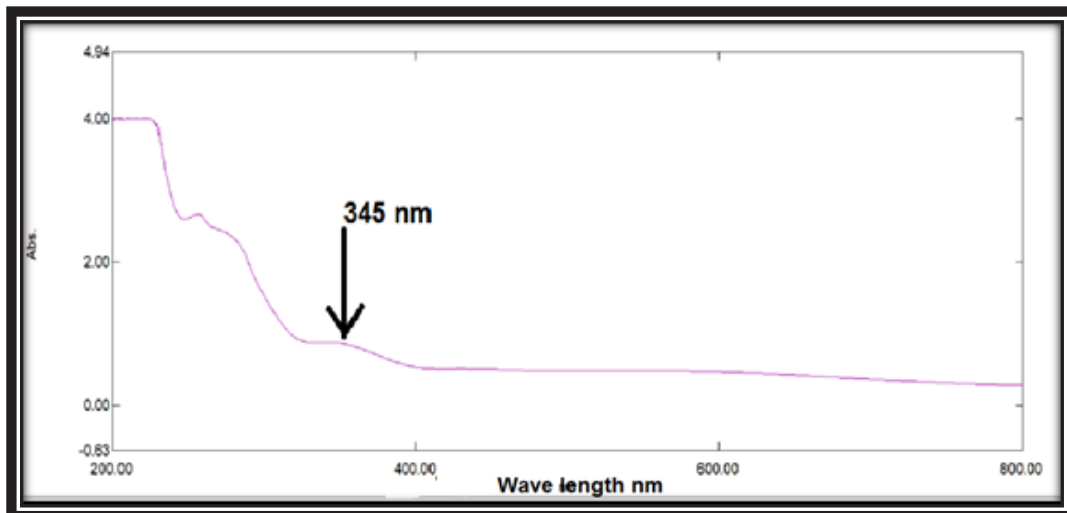


Fig. 2. UV/VIS absorption spectroscopy of silver nanoparticles (2 molar) synthesized from silver nitrate solution and hot aqueous extract of *R. rosemary* leaves.

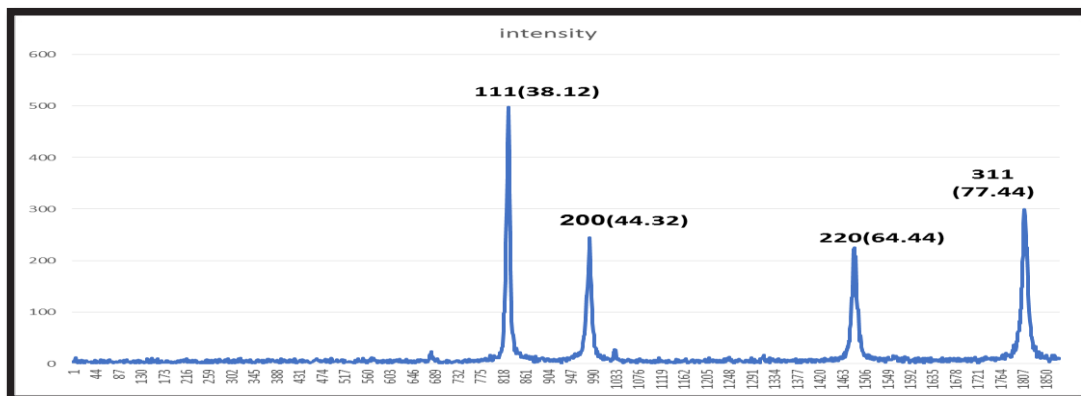


Fig. 3. X- of nanoparticles synthesized from aqueous extract of *R. officinalis* leaves.

of 1.9 cm with *A. niger* and the lowest inhibition rate was 35.5% with a growth rate of 5.8 cm with *F. verticillioides* as shown in Table 1 and Fig. 10.

It is clear from the results that the synthesis of particles from plant extracts is an easy, important and low-cost method, and that the color change of the solution is due to the reduction processes that occurred, which is due to the presence of active compounds or secondary metabolites present in the extracts such as organic and amino acids, proteins, sugars, flavonoids and phenols that are abundant, as well as alkaloids [16], and these compounds play an important role in reduction, stabilization and stabilization processes [17]. Increasing the incubation time also plays a significant role in increasing the reduction processes [18]. The change in color refers to the surface plasmon phenomenon [19].

UV/Vis/UV absorption spectroscopy for the

initial characterization of formed nanoparticles is also very important and is a practical and reliable technique. UV-visible spectroscopy evaluates the synthesis and stability of AgNPs, the characteristic optical properties of AgNPs make them highly reactive to certain wavelengths of light [4]. Due to the surface plasmon resonance (SPR) phenomenon, AgNPs exhibit good absorption in the visible spectrum in the range of 200-800 nm [20].

In order to evaluate both the molecular and crystalline structure of a compound, XRD is a very valuable analytical technique. During XRD analysis, a beam of X-rays is projected onto the crystal and the atoms are scattered, resulting in the formation of diffraction patterns, and the interference of the scattered X-rays according to Bragg's law can be used to determine the polycrystalline properties of the crystal or polycrystalline material

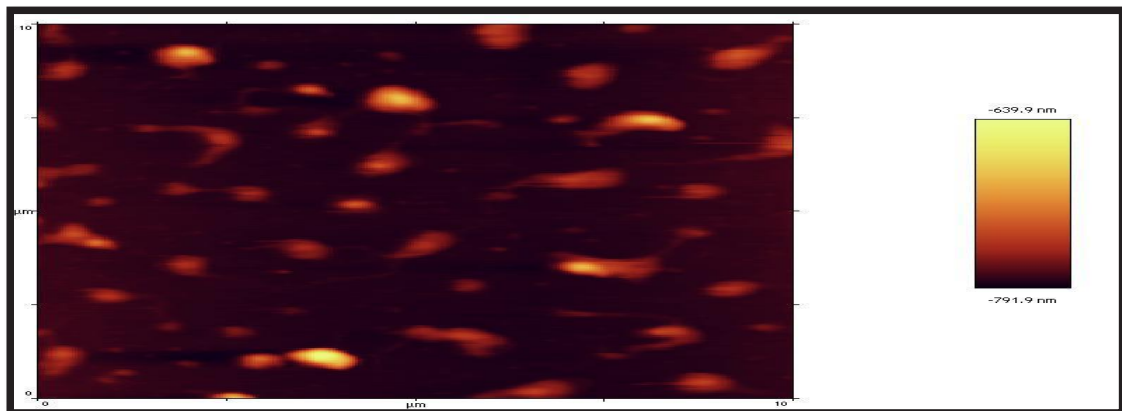


Fig. 4. image of the surface of biosynthesized silver nanoparticles by AFM (Two dimensional).

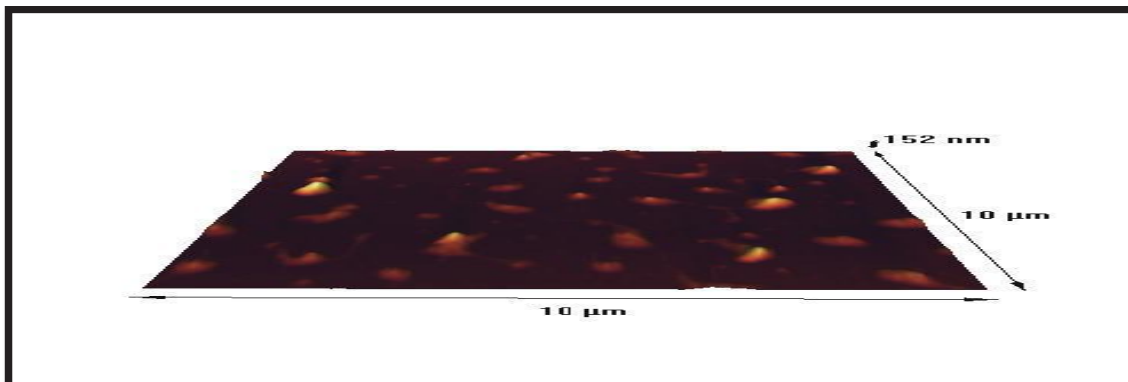


Fig. 5. image of the surface of biosynthesized silver nanoparticles by AFM (Three dimensional).

[21,22,23]. Some non-specific peaks were also observed, which may be due to the bio-organic phase such as metalloproteins on the surface of silver nanoparticles [24]. Or it may be due to a few biomolecules such as enzymes or proteins in the plant extract [25]. Scanning electron microscopy (SEM) is a surface imaging tool used to analyze various particle sizes, size distributions, shapes of nanomaterials, and surface morphology of particles synthesized at the nanoscale [26]. Large nanoparticles were observed due to aggregation and aggregation was probably caused by the

presence of some active plant components on the surface of the nanoparticles acting as a capping agent [27].

In recent years, many studies and researches have indicated the importance of using nanomaterials in the control and treatment of pathogenic microorganisms, including fungi. The widespread use of silver nanoparticles is due to their unique physical and chemical antimicrobial properties, with a myriad of applicable activities in various fields, including the pharmaceutical industry [9]. Rao and Paria [28] used silver

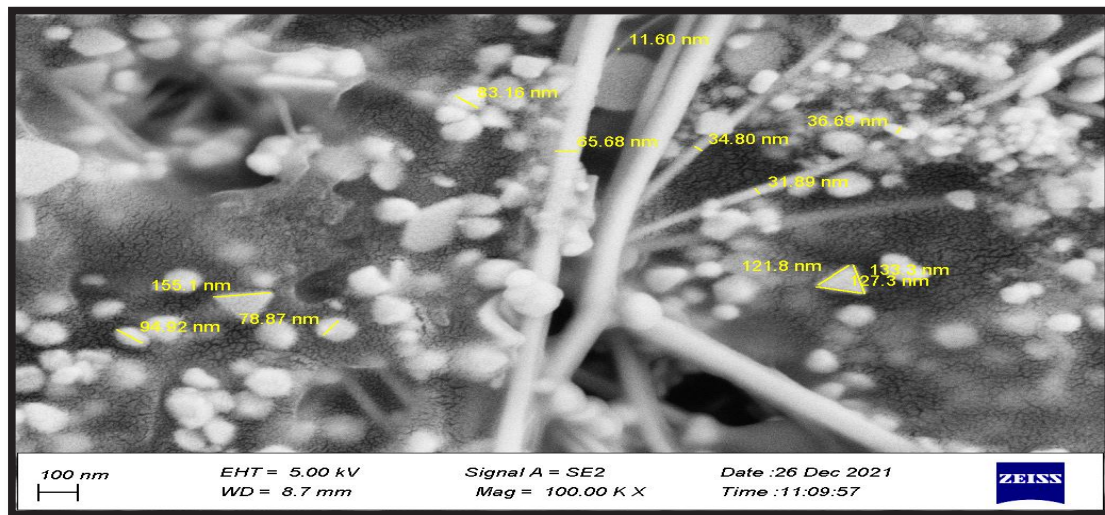


Fig. 6. Biosynthesized silver nanoparticles by SEM microscope at 100 nm.

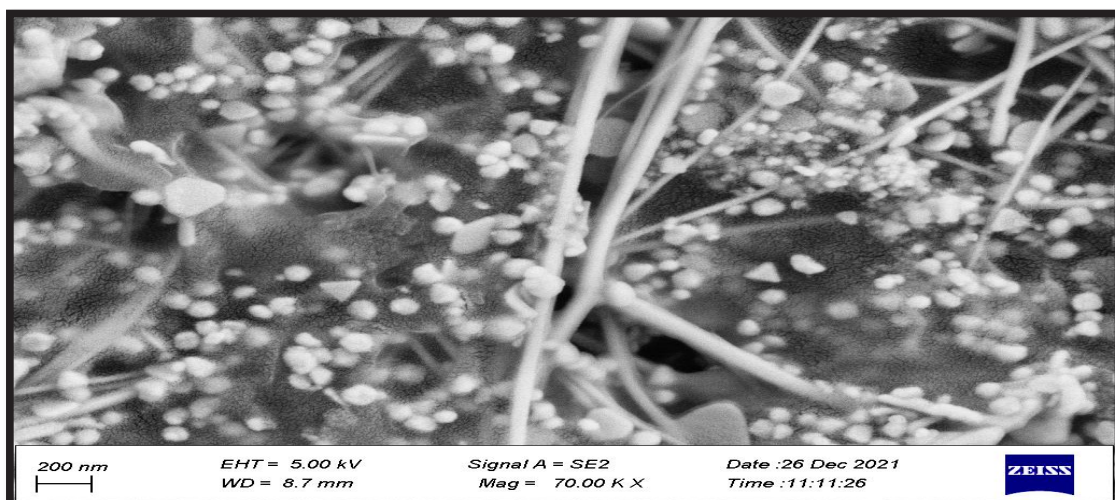


Fig. 7. Biosynthesized silver nanoparticles under SEM microscope at 200 nm.

nanoparticles synthesized in the laboratory as a fungicide against the fungus *F. He*. He proved that they can be used as an effective pesticide and can also protect several plants, including tomatoes, potatoes, apples, grapes and others from pathogenic fungi that infect these plants as they work to analyze and destroy the molecules that enter the cell wall synthesis or inhibit the process of cell wall synthesis as well as the destruction of the cell membrane of fungal cells and thus

disrupt the balance of the fungal cell, leading to its death. Yelaet *al.* [29]. Silver nanocomposites and some oils had a high inhibitory role against some fungi, including *F. oxysporum*. As for the possible mechanisms of how nanoparticles affect the cells of microorganisms, Prasheret *al.* [30] mentioned that the possible mechanism by which nanoparticles inhibit and kill fungal colonies is that due to the small size of these nanoparticles, they can penetrate the cell wall and produce free

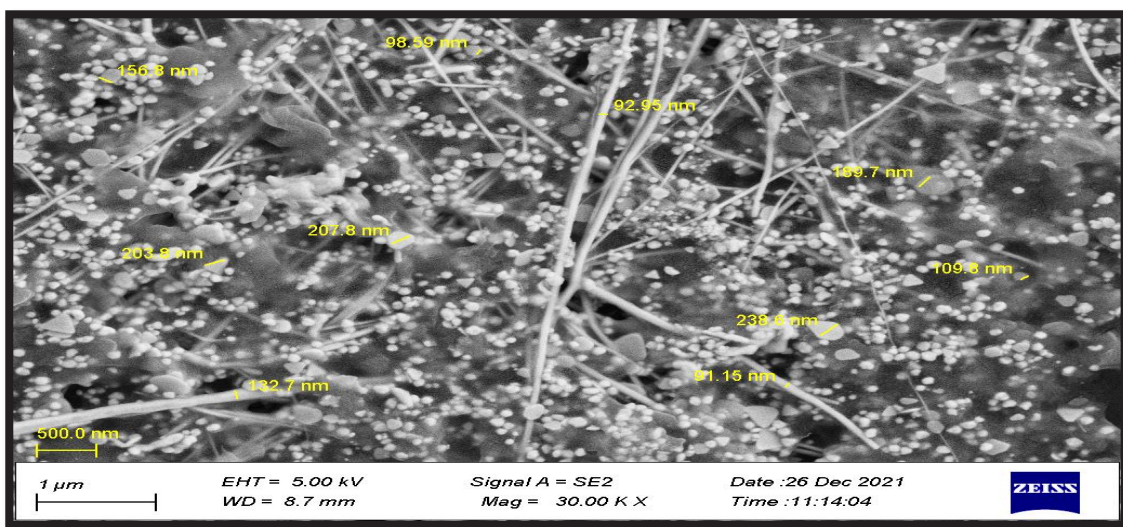


Fig. 8. Biosynthesized silver nanoparticles under SEM microscope at 1 μm .

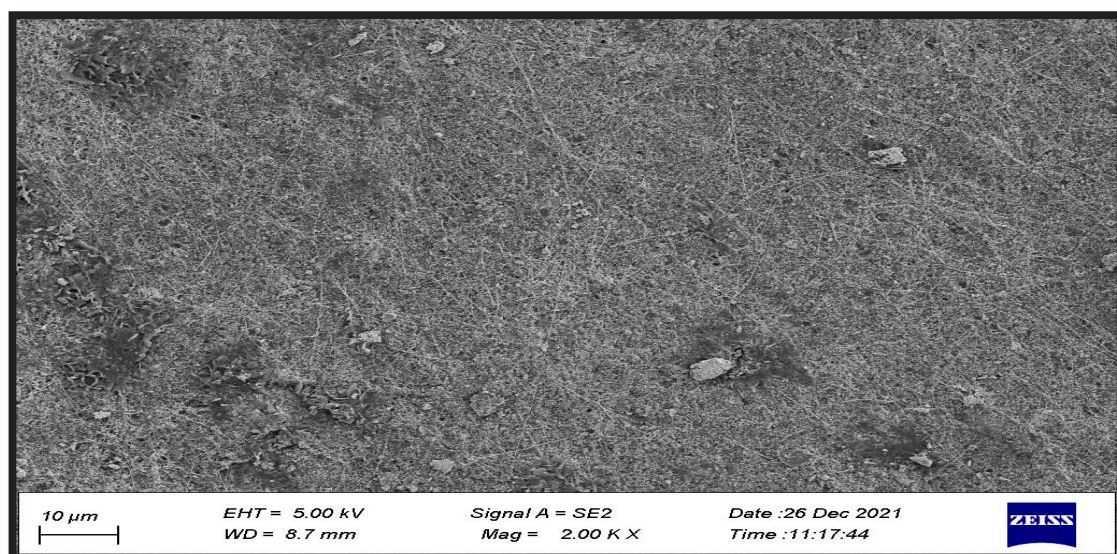


Fig. 9. Biosynthesized silver nanoparticles under SEM microscope at 10 μm .

Table 1. Effect of AgNPson some fungi producingmycotoxin.

| Species | Inhibition % after 7 days | | | Fungicide Tachigaren | Control |
|---------------------------|---------------------------|---------|-----------|----------------------|---------|
| | 50ppm\L | 75ppm\L | 100 ppm\L | | |
| <i>A.niger</i> | 78.8 | 88.8 | 100 | 100 | 0.0 |
| <i>F. verticillioides</i> | 35.5 | 36.6 | 73.3 | 84.4 | 0.0 |
| <i>F. solani</i> | 45.5 | 71.1 | 100 | 100 | 0.0 |
| L.S.D (0.05) | | | 0.102 | | |

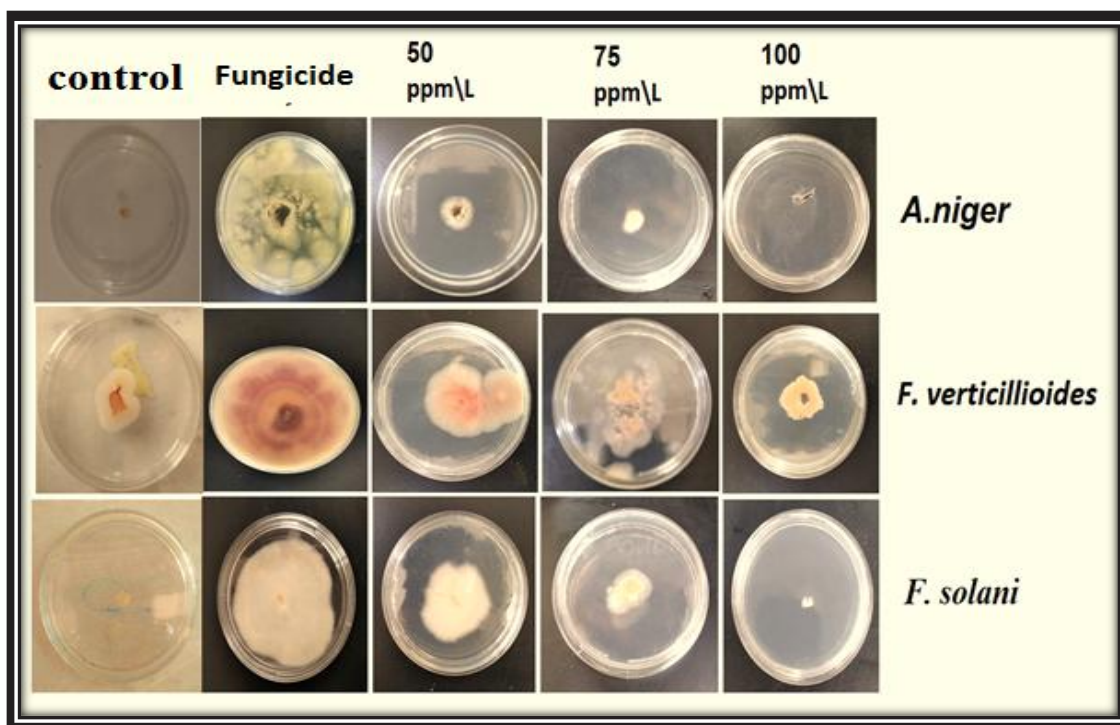


Fig. 10. Effect of AgNPs on some fungi producing mycotoxin.

radicals and thus lead to programmed cellular death.

CONCLUSION

The study confirmed that the biosynthesis of nanoparticles through the use of plant extracts is a safe, fast, inexpensive and effective method. The study showed that the use of biosynthesized silver nanoparticles can be used as a fungicide for pathogenic and mycotoxin-producing fungi, as the inhibition rate reached 100%.

CONFLICT OF INTEREST

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

manuscript.

REFERENCES

1. Goyal S, Kumar S. Anti-Anxiety Activity Studies of Various Extracts of Pulsatilla Nigricans Stoerck. International Journal of Pharmaceutical Sciences and Drug Research. 2010;291-293.
2. Mahmoodnia L, Aghadavod E, Beigrezaei S, Rafeian-Kopaei M. An update on diabetic kidney disease, oxidative stress and antioxidant agents. Journal of Renal Injury Prevention. 2017;6(2):153-157.
3. Yassin SG, Al-Khafaji MF, Al-Sgheer SK. Molecular diagnosis and phytochemical analysis of alcoholic extracts of Moringa oleifera leaves by GC-MS. International Journal of Biology Sciences. 2024;6(1):28-37.
4. A.S S, A E-S. Taxonomy Study of Some Members of Lamiaceae Through Morphological Traits and Effects on General Condition of the Honey Bee (*Apis mellifera* L.). International

- Journal of Pure and Applied Zoology. 2019;7(4).
- Zhang X-F, Liu Z-G, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *Int J Mol Sci*. 2016;17(9):1534.
 - Veerasingam R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N, et al. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society*. 2011;15(2):113-120.
 - Azarbani F, Shiravand S. Green synthesis of silver nanoparticles by *Ferulago macrocarpa* flowers extract and their antibacterial, antifungal and toxic effects. *Green Chemistry Letters and Reviews*. 2020;13(1):41-49.
 - Kouvaris P, Delimitis A, Zaspalis V, Papadopoulos D, Tsipas SA, Michailidis N. Green synthesis and characterization of silver nanoparticles produced using *Arbutus Unedo* leaf extract. *Mater Lett*. 2012;76:18-20.
 - Kasim S, Dali S, Rahmah M. Synthesis of silver nanoparticles using bioreductors from clove leaf extract (*Syzygium aromaticum*) and test of its antibacterial activity. *Journal of Physics: Conference Series*. 2021;1763(1):012051.
 - Bamal D, Singh A, Chaudhary G, Kumar M, Singh M, Rani N, et al. Silver Nanoparticles Biosynthesis, Characterization, Antimicrobial Activities, Applications, Cytotoxicity and Safety Issues: An Updated Review. *Nanomaterials*. 2021;11(8):2086.
 - Richardson PM, Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Second Edition. Brittonia. 1990;42(2):115.
 - Labanni A, Zulhadjri Z, Handayani D, Ohya Y, Arief S. The effect of monoethanolamine as stabilizing agent in *Uncaria gambir* Roxb. mediated synthesis of silver nanoparticles and its antibacterial activity. *J Dispersion Sci Technol*. 2019;41(10):1480-1487.
 - Maciel MVdOB, Almeida AdR, Machado MH, Melo APZd, Rosa CGd, Freitas DZd, et al. *Syzygium aromaticum*; L. (Clove) Essential Oil as a Reducing Agent for the Green Synthesis of Silver Nanoparticles. *Open Journal of Applied Sciences*. 2019;09(02):45-54.
 - Sumi Maria B, Devadiga A, Shetty Kodialbail V, Saidutta MB. Synthesis of silver nanoparticles using medicinal *Zizyphus xylopyrus* bark extract. *Applied Nanoscience*. 2014;5(6):755-762.
 - Latha P, Sudhakar P, Sreenivasulu Y, Naidu PH, Reddy PV. Relationship between total phenols and aflatoxin production of peanut genotypes under end-of-season drought conditions. *Acta Physiologiae Plantarum*. 2007;29(6):563-566.
 - Raut RW, Kolekar NS, Lakkakula JR, Mendhulkar VD, Kashid SB. Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre. *Nano-Micro Letters*. 2010;2(2):106-113.
 - Vanaja M, Annadurai G. *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. *Applied Nanoscience*. 2012;3(3):217-223.
 - Saliem AH, Ibrahim OMS, Salih SI. Biosynthesis of Silver Nanoparticles using Cinnamon *Zeylanicum* Plants Bark Extract. *Kufa Journal For Veterinary Medical Sciences*. 2016;7(1):51-63.
 - Skandalis N, Dimopoulou A, Georgopoulou A, Gallios N, Papadopoulos D, Tsipas D, et al. The Effect of Silver Nanoparticles Size, Produced Using Plant Extract from *Arbutus Unedo*, on Their Antibacterial Efficacy. *MDPI AG*; 2017.
 - Vijayaraghavan K, Ashokkumar T. Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. *Journal of Environmental Chemical Engineering*. 2017;5(5):4866-4883.
 - Das R, Nath SS, Chakdar D, Gope G, Bhattacharjee R. Synthesis of silver nanoparticles and their optical properties. *J Exp Nanosci*. 2010;5(4):357-362.
 - Waseda Y, Matsubara E, Shinoda K. *X-Ray Diffraction Crystallography*. Springer Berlin Heidelberg; 2011.
 - Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity. *Chemico-Biological Interactions*. 2017;273:219-227.
 - Kulothungan L, Ramaswamy UMA. Green synthesis and characterization of silver nanoparticles using aqueous extract of *Marsilea minuta* Linn and evaluation of its antioxidant and antibacterial activities. *International Journal of Pharma and Bio Sciences*. 2018;9(1).
 - Kothekar D. Rapid synthesis of copper and silver nanoparticles using *Zingiber officinale* dried rhizome extract. *International Journal of Pharma and Bio Sciences*. 2017;8(3).
 - Probing Nanogap-Dependent Plasmonic Coupling in Gold Nanoparticle Superlattices by Scanning Tunneling Microscopy Induced Light Emission. *American Chemical Society (ACS)*.
 - Characterization and Antimicrobial Study of Nickel Nanoparticles Synthesized from *Dioscorea* (Elephant Yam) by Green Route. *International Journal of Science and Research (IJSR)*. 2015;4(11):216-219.
 - Rao KJ, Paria S. Use of sulfur nanoparticles as a green pesticide on *Fusarium solani* and *Venturia inaequalis* phytopathogens. *RSC Advances*. 2013;3(26):10471.
 - Yela AV, Jimenez VJ, Rodriguez DV, Quishpe GP. Evaluation of the Antifungal Activity of Sulfur and Chitosan Nanocomposites with Active Ingredients of *Ruta graveolens*, *Thymus vulgaris* and *Eucalyptus melliodora* on the Growth of *Botrytis fabae* and *Fusarium oxysporum*. *Biology and Medicine*. 2016;08(03).
 - Prasher P, Singh M, Mudila H. Oligodynamic Effect of Silver Nanoparticles: a Review. *BioNanoScience*. 2018;8(4):951-962.