Extracellular biosynthesis of sliver nanoparticles from some species of nematode trapping fungi

Cite as: AIP Conference Proceedings **2290**, 020036 (2020); https://doi.org/10.1063/5.0029423
Published Online: 04 December 2020

Anfal A. Lafta, and Ali A. Kasim

Extracellular Biosynthesis of Sliver Nanoparticles from Some Species of Nematode Trapping Fungi

Anfal A. Lafta[a)] and Ali A. Kasim

Biology Department, College of Science, University of Misan, Iraq

[a)] Corresponding author: alimycol@uomisan.edu.iq

Abstract. The objective of this study was to estimate the ability of seven species nematode trapping fungi isolated from Iraqi agriculture soil to biosynthesize silver nanoparticles. All tested species showed the ability to produce silver nanoparticles. The UV-Vis spectra obtained from the fungal free-cell (FCF) amended with AgNO3 at wavelengths 433.5-450 nm after 72hrs. showed significant variations in spectra of silver nanoparticles synthesis and different absorption peaks in specific wavelengths and the diameters of nanoparticles also differed according to the tested fungi. The absorption value of the tested fungi was between 0.3-0.5. Scanning electron microscope (SEM) micrographs showed that the synthesized silver nanoparticles are dispersed and mostly having spherical and ovoid shape within the size range between 5-90 nm. A.eudermata gave the smallest nanoparticles size of 5-35 nm, while A.cookedichison gave the largest nanoparticles size of 10-65 nm. Other species revealed different size of nanoparticles.

Keywords: Nematode-trapping fungi, Arthrobotrys oligospora, Sliver nanoparticles, Extracellular Biosynthesis, SEM micrographs.

INTRODUCTION

Nanotechnology provides mechanisms for the production, manipulation and use of small particles with size less than micron or not exceeding 100 nanometers to that of individual atoms (10-9), which lead to the rearrangement of atoms, resulting in the construction of new particles with new specifications that differ from the bulk materials (Kannan and Subbalaxmi, 2011; Moharrer et al., 2012). The last years have witnessed a significantly research interests in nanobiotechnology to find sources from nature for biosynthesis of metal nanoparticles as agents broadly used in multidisciplinary fields including medicine, agriculture, environment and industry (Marambio-Jones and Hoek, 2010; Kashyap et al., 2013). Among various biological systems used for synthesis, fungi are considered as the better ones due to its diversity and could control growth (Saha et al., 2010; Saxena et al., 2014). Fungi are natural source for production of many bioactive materials that can be applied in pharmaceutical and medical applications (Demain and Sanchez, 2009). Moreover, they can synthesize nanoparticles both extra and intracellularly, but nanoparticle extracellularly is appropriate for simpler downstream processing and handling of biomass (Saxena et al., 2014). Gade et al. (2008) showed that Aspergillus sp. were able to synthesis of AgNPs extracellularly.

Nematophagous fungi are a various group of fungal species that use their spores or mycelial structures to capture their prey. In addition, a large group of opportunistic fungi can parasitize the eggs and cysts of these nematodes (Niu et al., 2010). They can be used as a biological control agent for suppress the populations of plant-parasitic nematodes (Nordbring-Hertz et al., 2006). The synthesis and characterization of nanoparticles using nematophagous fungi have been reported (Wang, et al., 2012; Costa Silva et al., 2017). A continuation of research to discover more effective fungi for biosynthesis of silver nanoparticles, however, it is worth mentioning that there is no scientific report of the production of AgNPs using other species of nematode-trapping fungi. the present study therefore, aimed to examine some species of these the fungi for their abilities for synthesis of silver nanoparticles.

The 8th International Conference on Applied Science and Technology (ICAST 2020)
AIP Conf. Proc. 2290, 020036-1–020036-8; https://doi.org/10.1063/5.0029423
Published by AIP Publishing. 978-0-7354-4020-3/$30.00

MATERIALS AND METHODS

Fungal isolation and identification

The nematode-trapping fungi (Arthrobotrys conoides, A.cookedichison, A.eudermata, A.microscaphoides, A.oligospora, A.thaumasia, C.rosea were isolated from agriculture soil samples collected from Miasan province (southern Iraq) using sprinkling technique according to Jansson and Jaffee, 1990. The isolated fungi were identified according to Zhang and Hyde, (2014). Pure cultures were prepared using corn meal agar (CMA). The broth cultures of the tested fungi were prepared as follows: one disc (6 mm diameter) was cut from the axenic fungal culture (7 days) and inoculated into 250 ml Erlenmeyer flasks containing 100 ml of corn meal broth (CMB) (pH=6.4) at 25±1ºC,180 rpm for 10 days; Mycelia were separated from the culture broth by filtration on Whatman filter paper No.1 and washed three times with sterilized Milli-Q deionized water to remove the medium component from the mycelia biomass.

Biosynthesis of silver nanoparticles (AgNPs)

The fungal mycelia (10 gm wet weight) was mixed with 100 ml deionized water in Erlenmeyer flask (250 ml) and agitated in water bath at 120 rpm for 72 h. at 25 °C. The mixture was filtered by Whatman filter paper No.1 to get fungal free cell filtrate (FCF), 0.017 gm of AgNO3 was added to 100 ml of FCF and incubated at room temperature at dark condition to get a final concentration of 1 mM. Flaks containing FCF without AgNO3 were used as control. Triplicate flasks of the treated and untreated fungal filtrate were used in this study.

Detection of silver nanoparticle by UV-Visible spectrophotometric

The biosynthesized silver nanoparticles (AgNPs) within the FCF was examined by changing of color from pale yellow to dark brown during 24 hours and was further confirmed by UV-Vis spectrophotometer. 3 mL of FCF was taken after 72 hrs, then monitored using UV-Vis spectrophotometer at the wave lengths of 300 to 800 nm. Untreated free- cell filtrate was used as a control.

Scanning Electron Microscopic (SEM) analysis

The FCF treated with $AgNO_3$ was measured by using Scanning Electron Microscopic (SEM) (Germany Fesem-Zies) The results were recorded in Shahid Beheshti University, Tehran, Islamic Republic of Iran.

RESULTS

The formation of AgNPs was appeared due to the change in the color of the broth, which converted to yellowish brown (24 hrs) after the addition of the AgNO3 solution to the fungal filtrate.

The UV-Vis spectra obtained from the fungal free-cell (FCF) amended with AgNO3 at wavelengths 300-800 after 72hr. showed significant variations in spectra of silver nanoparticles synthesis and different absorption peaks (433.5-450 nm) in specific wavelengths. Moreover, diameters of nanoparticles also differed according to the tested fungi. Moreover, SEM images showed that the shape of biosynthesized AgNPs by the tested fungi were mostly spherical or/and ovoid and dispersed with 5-90 nm dimeters.

A.conoides showed maximum absorption at 448 nm wavelength with a absorption value of 0.5, SEM micrographs showed that the silver nanoparticles are dispersed or aggregated and mostly appeared as spheroidal shape and their diameter ranging between 15-90 nm (Fig. 1). The absorption value reached to 0.3 at maximum absorption 447 nm wavelength were appeared when applying A.cookedichison filtrate with a large number of dispersed and ovoid nanoparticles shape with 10-65 nm diameter (Fig 2). On the other hand, A.eudermata revealed maximum absorption at 449.5 nm wavelength and absorption value was 0.4. Moreover, image of SEM showed a few number of small (5-35 nm diameter) spherical and ovoid nanoparticles (Fig. 3). Figure 4 showed the ability of A.microscaphoides to produce nanoparticles which gave dispersed, few, small and often spherical nanoparticles and diameter ranging between 10-50 nm and the maximum absorption at wavelength 450 nm and an absorption value equal to 0.3. The maximum absorption of AgNPs synthesized by A.oligospora was at a wavelength of 433.5 nm with an absorption value of 0.3. AgNPs was dispersed and mostly appeared as spherical and ovoid nanoparticles with diameter ranging

between 10-80 nm.(Fig. 5). AgNPs synthesized by A.thaumasia revealed the maximum absorption at 435 nm wavelength and 0.3 absorption value, SEM micrograph showed aggregated nanoparticles and mostly appeared as ovoid and spherical shape with diameter ranging between (5-90) nm. (Fig.6). Finally, C.rosea showed the maximum absorption at 448.5 nm wavelength and absorptive value reached to 0.5, while the silver nanoparticles were aggregated, ovoid and 8-55 nm diameter (Fig.7). FCF without AgNO3 (control) showed no absorption at 300-800 nm wavelength and produced no nanoparticles.

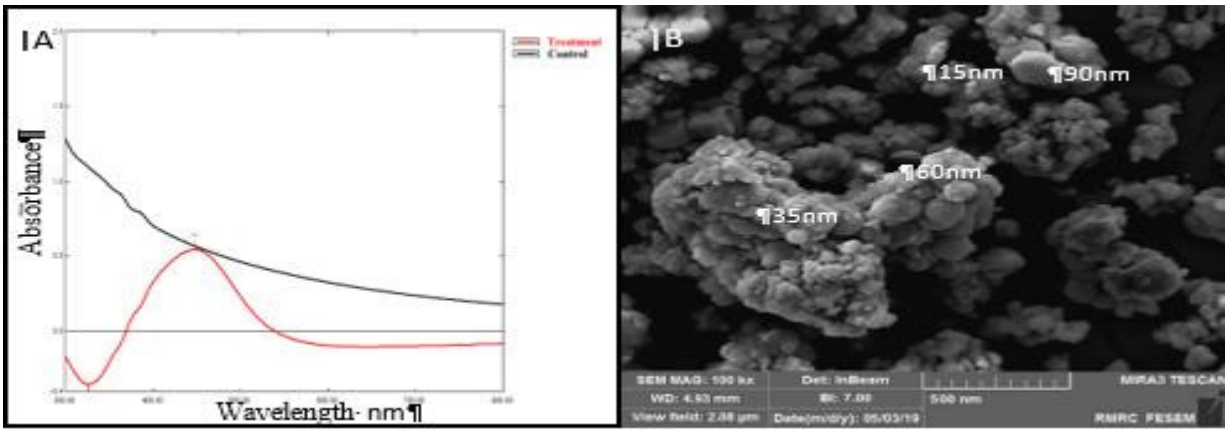

FIGURE 1. Fungal free-cell filtrate (FCF) of *A.conoides* A. UV-Vis spectrum of (FCF) containing silver nanoparticles(448 nm wavelength, 0.5 absorption value). B. SEM micrographs showing the silver nanoparticles appeared as spheroidal shape and 15-90 nm diameter at magnification 100 KX.

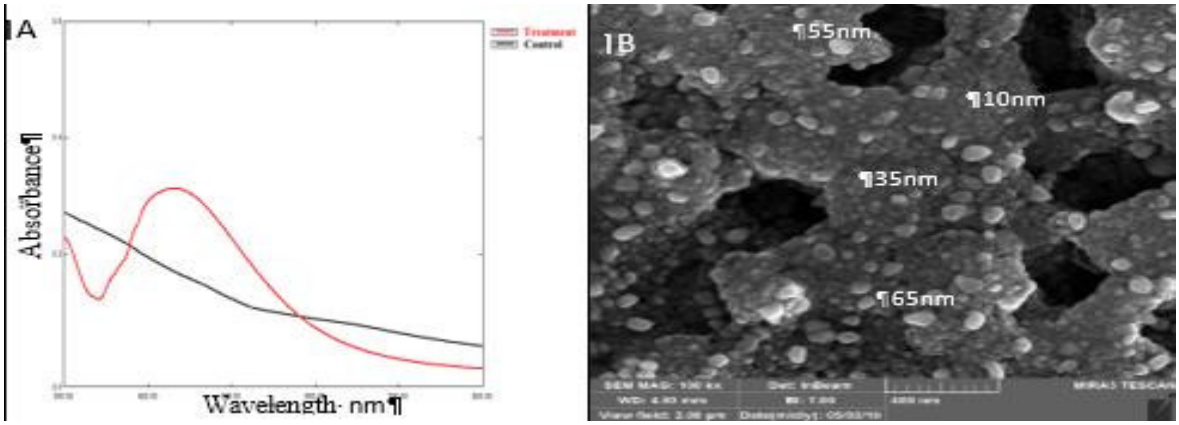

FIGURE 2. *A. cookedichison* FCF: A. UV-Vis showed maximum absorption at 447 nm wavelength and absorption value reached to 0.3. B. SEM image showed the silver nanoparticles in FCF which are a large number of dispersed and ovoid shape and 10-75 nm diameter at magnification (100 KX).

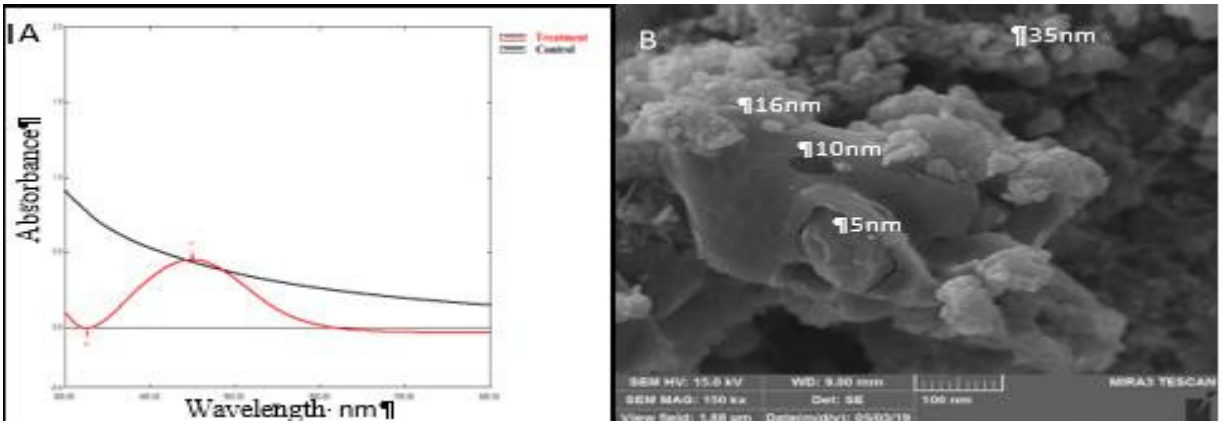

FIGURE 3. Filtrate of *A.eudermata* revealed maximum absorption at 449.5 nm wavelength, absorption value was 0.4., image of SEM showed a few number of small (10-75 diameter) spherical and ovoid nanoparticles (100 KX).

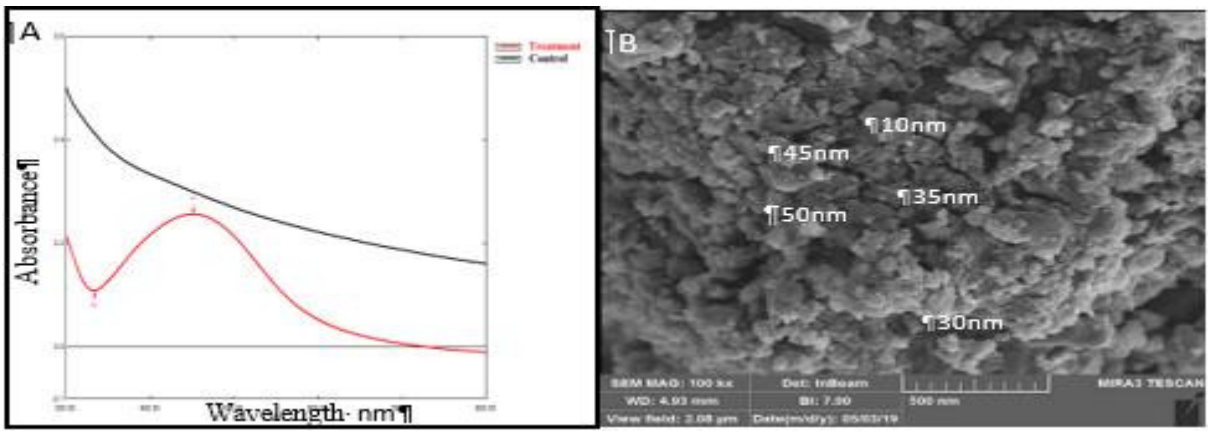

FIGURE 4. *A.microscaphoides* filtrate: A. UV-Vis spectrum of FCF containing silver nanoparticles, the maximum absorption at wavelength 450 nm and a absorption value equal to 0.3. B. SEM micrographs showing the silver nanoparticles spherical and diameter ranging between 15-65 nm (100 KX).

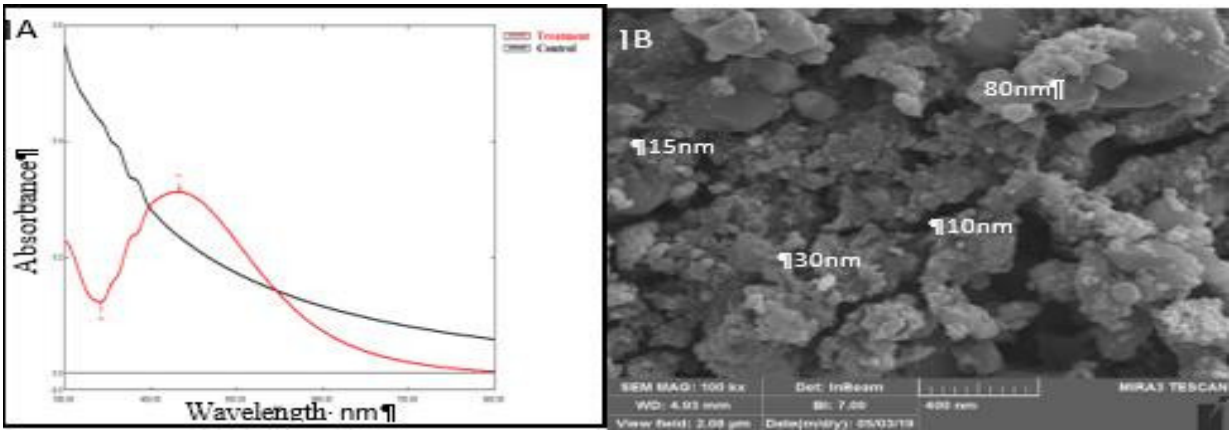

FIGURE 5. FCF of *A.oligospora* :A. The maximum absorption was at a wavelength of 433.5 nm and a absorption value was 0.3. B. SEM micrographs showing dispersed and spherical and ovoid nanoparticles and 10-75 nm diameter (100 KX).

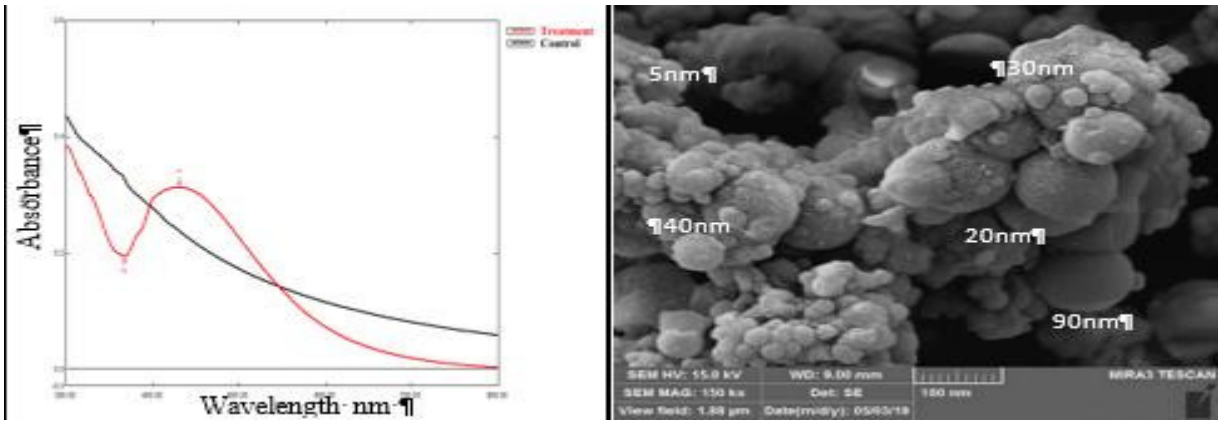

FIGURE 6. *A. thaumasia* filtrate: A. UV-Vis spectrum of FCF revealed the maximum absorption at 435 nm wavelength and 0.3 absorption value. B. SEM micrograph showed aggregated nanoparticles and ovoid and spherical shape (20-95nm diameter) (100 KX).

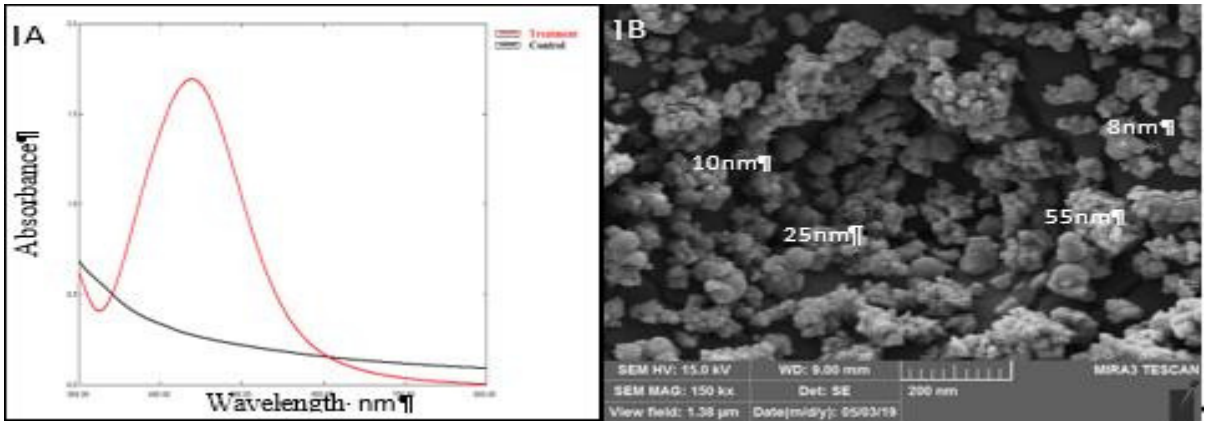

FIGURE 7. Filtrate of *C.rosea*: A. showed the maximum absorption at 448.5 nm wavelength and absorptive value reached to 0.5, B. the silver nanoparticles were aggregated, ovoid and 10-65nm diameter (100 KX).

DISCUSSION

Microorganisms such as fungi have important potential for the formation of silver nanoparticles (AgNPs) for many applications (Alghuthaymi *et al*., 2015). The present study showed that the tested nematode trapping fungi exhibited a high potentiality for biosynthesis of silver nanoparticles in culture medium as indicated by the color change from yellow into dark brown after 24 hours of incubation after being treated with 1mM $AgNO_3$ solution and indicated that 1mM concentration of $AgNO_3$ is appropriate for the synthesis of AgNPs. These results are in agreement with other previous studies using different fungal species (Kathireshan *et al.,* 2009; Wang *et al*., 2012; Verma *et al.*, 2013; Neethu *et al.*, 2018). However, many studies reported that there are some variations in characterization of silver nanoparticles biosynthesized by different fungal species (Maliszewska *et al.*, 2009; Raheman *et al*., 2011; Chitra and Annadurai, 2013). These differentiations might be due to the source of fungal isolates or strains and culture conditions (Marambio-Jones and Hoek, 2012). Neethu *et al.* (2018) refer that the AgNP synthesis was increased with the fungal mycelium amount. Nematode trapping fungi use adhesive or mechanical hyphal traps to cutch nematode and these traps produce usually abundance, Wang *et al.* (2012) showed that secreted nanoparticles are not related to trap formation, but to the growth of mycelia.

Visual observation of tested fungal filtrates with $AgNO_3$ showed a color change from colorless to reddish-brown indicates the production of silver nanoparticles without a doubt and consider a clear indication of the synthesize of silver nanoparticles (Verma *et al.*, 2010). This conversion was taken place by nitrate reductase requiring NADH/NADPH as coenzyme (Kumar *et al*., 2007; Karbasian *et al*., 2008). Despite of the mechanistic that take part in fabrication of silver nanoparticles by fungi are still unclear, however, it has been proposed that biosynthesis of silver nanoparticles is associated with the enzyme reductase which is responsible for the reduction of Ag^+ ions and synthesis of AgNPs (Duran *et al.*, 2005). Furthermore, a reduction of Ag^+ may be attributed to a conjugation between the electrons shuttle with reductase enzyme involvement (Maliszewska *et al.*, 2009).

The present study revealed that UV-Vis spectrophotometry analysis of tested fungi solutions showed maximum absorption between 433.5-451.5 nm which differed the AgNPs synthesis by the examined fungi and a completed biosynthesis of AgNPs was after 72 h of free-cell filtrate incubation. AgNPs showed strong absorption of electromagnetic waves in the visible range due to the localized surface plasmon resonance (LSPR) (Costa Silva *et al.,* 2017). This phenomenon occurs due to collective oscillations of the NP conduction electrons by visible light irradiation (Basavaraja *et al*., 2008; Chitra and Annadurai, 2013). The production of NPs was established by UV–Vis spectroscopy due to the color of the colloidal silver is attributed to the specific LSPR (Costa Silva *et al*., 2017). The peaks of wavelengths were between 433.5-451.5 nm in the UV, Ninganagouda *et al.* (2013) attribute this to a wavelength range characteristic of the localized surface plasmon resonance (LSPR) were performed on a UV–Vis spectrophotometer of AgNPs.

The morphology and size of biosynthesized silver nanoparticles in solution depend on different reaction parameters. The present study revealed that the shape of silver nanoparticles was spherical or/and ovoid with 5-90 nm dimeters. Many studies reported a variable shape and size of silver nanoparticles synthesized depending of fungal species, pH and temperature (Martinez-Castanon *et al.*, 2008; Marambio-Jones and Hoek, 2010; Muhsin and Hachim, 2015).

Matrinez-Castanon *et al.,* (2008) showed that the absorption spectrum of spherical shape of silver nanoparticles present a maximum between 420-450 nm. However, our study agreed with the above mentioned.

CONCLUSION

A conclusion can be derived that the tested selected nematode trapping fungi revealed a high potentiality for synthesis of silver nanoparticles and this result is recorded for the first time for some of these fungi.

Acknowledgment

The authors are thankful to University of Misan (Iraq) for providing financial support.

REFERENCES

1. Alghuthaymi MA, Almoammar H, Rai M, Said-Galiev E, and Abd-Elsalam KA.(2015). Myconanoparticles: synthesis and their role in phytopathogens management. Biotechnol Biotechnol Equip. 29(2): 221–236.
2. Basavaraja S, Balajia SD, Arunkumar Lagashettyc, Rajasab AH, and Venkataramana A. (2008). Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. *Mater Res Bull*. 43:1164–1170.
3. Chitra K and Annadurai G (2013). Bioengineered silver nanobowls using Trichoderma viride and its antibacterial activity against Gram-positive and Gram-negative bacteria. Journal of Nanostructure Chemistry. 39: 3-9.
4. Costa Silva LP, Oliveira JP, Keijok WJ, da Silva AR, Aguiar AR, Guimarães M, Ferraz CM, Araújo JF, Tobias FL and Braga FR. (2017). "Extracellular Biosynthesis of Silver Nanoparticles Using the Cell-Free Filtrate of Nematophagous Fungus *Duddingtonia flagrans*." *International Journal of Nanomedicine* 12: 6373–81.
5. Demain AL and Sanchez S. (2009). Microbial drug discovery: 80 years of progress. Journal of Antibiotics, 62, 5-16.

6. Duran N., Marcato PD, Alves OL, Desouza, G and Esposit OE. (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. Journal of Nanobiotechnology, 3, 1–8.
7. Gade A, Bonde PP, Ingle AP, Marcato P, Duran N, and Rai MK. (2008) Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. Journal of Bio based Materials and Bioenergy, 2(3): 1–5.
8. Kannan, N. and Subbalaxmi, S. (2011) Biogenesis of nanoparticles - a current. Rev. Adv. Mater. Sci. 27:99-114.
9. Karbasian, M., Atyabi, S.M., Siadat, S.D., Momen, S.B. and Norouzian, D. (2008). Optimizing Nano-silver Formation by *Fusarium oxysporum* PTCC 5115 Employing Response Surface Methodology. *Am. J. Agri. & Biol., 3(1): 433-437.*
10. Kashyap, P.L., Kumar, S., Srivastava, A.K., and Sharma, A.K. (2013). Myconanotechnology in agriculture: a perspective. World Journal of Microbiology and Biotechnology, 29, 191-207.
11. Kathireshan K, Manivannan S, Nabeel MA and Dhivya B. (2009). Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment, Colloids Surfaces B Biointerfaces. 71: 133–137.
12. Kumar S.A, Abyaneh, M.K., Gosavi, S.W., Kulkarni, S.K., Pasricha, R., Ahmad, A. and Khan, M.I. (2007). Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnol Lett 29: 439-445.
13. Maliszewska I., Szewezk K., and Waszak K. (2009). Biological synthesis of silver nanoparticles. Journal of Physics, 146, 1-6.
14. Marambio-Jones C. and Hoek, EMV. (2010). A review of the antibacterial effects of silver nanomaterilas and potential implications for human health and the environment. Journal of Nanoparticles Research, 12,1531-1551.
15. Matrinez-Castanon GA, Nino-Martinez N, Matinez-Gutierrez F., Martinez-Mendoza, JR, and Ruiz F. (2008). Synthesis and antibacterial activity of silver nanoparticles with different sizes. Journal of Nanoparticles Research, 10, 1343-1348.
16. Moharrer, S. Mohammadi, B. Gharamohammadi, RA. and Yargoli M. (2012). Biological synthesis of silver nanoparticles by *Aspergillus flavus*, isolated from soil of Ahar copper mine. Indian J. Sci. & Tech. 5(53), 2443-2444.
17. Muhsin TM. and Hachim AK. (2015). Characterization and antibacterial efficacy of silver nanoparticles biosynthesized by the soil fungus *Curvularia tuberculata*. NanoScience Technology, 1, 5-11.
18. Neethu S. Midhun SJ. Radhakrishnan EK. and Jyothis M. (2018). Green synthesized silver nanoparticles by marine endophytic fungus *Penicillium polonicum* and its antibacterial efficacy against biofilm forming, multidrug-resistant *Acinetobacter baumanii*, Microb Pathog. 116:263-272.
19. Ninganagouda S. Rathod V. Jyoti H. Singh D. Prema K. and Haq MU. (2013). Extracellular biosynthesis of silver nanoparticles using *Aspergillus flavus* and their antimicrobial activity against gram negative MDR strains. *Int J Pharma Bio Sci*. 4(2):222–229.
20. Niu XM. Wang YL., Chu YS, Xue HX, Li N, Wei LX, Mo MH and Zhang QK. (2010). "Nematodetoxic Aurovertin-Type Metabolites from a Root-Knot Nematode Parasitic Fungus *Pochonia chlamydosporia*." *Journal of Agricultural and Food Chemistry* 58 (2): 828–34. https://doi.org/10.1021/jf903259n.
21. Nordbring-Hertz B. Jansson HB, and Tunlid A. (2006). "Nematophagous Fungi." *John Wiley & Sons*, 1–11.
22. Raheman F, Deshmukh S, Ingle A, Gad A, and Raj M. (2011). Silver nanoparticles: Novel antimicrobial agent synthesized from an endophytic fungus *Pestalotia* sp. Isolated from leaves of *Syzygium cumini* L. Nanoscience and Biomedical Engineering, 3, 174-178.
23. Saha S, Sarkar J, Chattopadhyay D, Patra S, Chakraborty A, and Acharya K. (2010). Production of silver nanoparticles by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Dig J Nanomater Bios, 5(4): 887–895.
24. Saxena, J, Sharma MM, Gupta S, and Singh A. (2014). Emerging role of fungi in nanoparticle synthesis and their application. World Journal of Pharmacy and Pharmaceutical Science 3 (9): 1586–1613.
25. Verma VC, Kharwar RN and Gange AC. (2010). Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus Aspergillus clavatus., Nanomedicine (Lond).
26. Verma S, Abirami S, and and Mahalakshmi V. (2013). Anticancer and antibacterial activity of silver nanoparticles biosynthesized by *Penicillium* sp. and its synergistic effect with antibiotics. Journal of Microbiology and Biotechnology Researches, 3, 54-71.

27. Wang Y, Sun L, Yi S, Huang Y, Lenaghan YC, and Zhang M. (2012). Naturally Occurring Nanoparticles from *Arthrobotrys oligospora* as a Potential Immunostimulatory and Antitumor Agent. Adv. Funct. Mater. 1-10DOI: 10.1002/adfm.201202619.
28. Zhang, KQ, and Hyde KD. (2014). *Nematode-Trapping Fungi. Springer Dordrecht Heidelberg*. B.V., Dord. New York: Springer.