Identification of Fungal secondary Metabolites of *Isaria fumosorosea* by GC-Mass Analysis used for the green synthesis of Silver nanoparticles

Maryem Akbal Hasoon[1], Ahmed Saeed Mohmed[2] and Jamaal Hussein Kadhim[3]

[1]Al-Furat Al-Awsat Technical University, Najaf, Iraq.
[2]College of Agriculture, Al-Qasim Green University, Babil, Iraq.
[3]Faculty of Agriculture, University of Kufa, Najaf, Iraq.
Corresponding author email: ahmedsa@agre.uoqasim.edu.iq

DOI: https://doi.org/10.36077/kjas/2025/v17i1.11418

Received date: 9/3/2023

Accepted date: 6/5/2023

Abstract

Entomopathogenic fungi are considered biological control agents for insects, which have a safe and environmentally friendly role. The action of insect pathogenic fungi is not limited to their mechanism of action to produce spores. However, it also secretes secondary metabolites, which also have a role in insect pathogens and have recently been shown to have a role in the biosynthesis of nanoparticles. *Isaria fumosorosea* is one of the most important E. pathogens that also, secrete many secondary metabolites. The current study included the detection of secondary metabolites of *I. fumosorosea* by GC-Mass analysis and the possibility of using these metabolites as reducing agents in the synthesis of silver nanoparticles. Results revealed that the crude filter contains many compounds such as sorbitol, heptadecane, Z-5-nonadecene, and heneicosane through GC-Mass analysis. The results also indicated that the secondary metabolites were successful in the biosynthesis of silver nanoparticles the properties of which have been described by several methods such as UV-Vis spectrophotometer where The absorption accrues at 419 nm. Moreover, the field emission scanning electron microscope (FESEM) explained the size and shape of Ag NPs where the range size was 44.66 -89.23 nm with roughly circular or spherical shape. In conclusion, the crude filtrate of *I. fumosorosea* contains many secondary metabolites compounds which showed effectiveness in the biosynthesis of AgNPs.

Keywords: *Isaria fumosorosea*, GC–Mass , Ag NPs

Introduction

With the increasing need to search for safe and environmentally friendly means as an alternative to chemical insecticides which cause harm to humans, animals, and the environment, Entomopathogenic fungi have proven their ability to control insect pests as one of the biocontrol agents (13). *Isaria fumosorosea* is one of the most important Entomopathogenic fungi which showed effectiveness in controlling insect pests through some mechanisms, such as the production of spores (7). There are other mechanisms for the action of fungi such as toxins, and enzymes, as secondary metabolites of entomopathogenic, which play a role in controlling insects (2). in addition to, A new role for secondary metabolites of entomopathogenic fungi has recently emerged. It is a reducing agent in biosynthesis nanoparticles (12), where Nanoparticles showed great effectiveness in the agricultural field especially controlling different insect pests (4),(9) . The use of secondary metabolites of fungi by researchers comes due to their high efficiency in the process of synthesis of nanoparticles, ease of obtaining them, and cheap price, as well as the safety of the environment and workers in the process of preparing nanoparticles. (10). There have been reports of many genera of fungi that produce silver nanoparticles such as *Lecanicillium lecanii* (5) , Trichoderma (16), Aspergillus (11), Fusarium (17), Penicillium (14). The focus on selecting silver nanoparticles in biosynthesis for several reasons, including superiority over other nanoparticles in terms of their biological, chemical and physiological properties. They are also very important due to their well-regulated size, composition and crystalline nature, low cell toxicity, excellent thermal stability and low volatility (6). Our current study aims to use GC-Mass technology to detect the active compounds extracted from the crude filtrate plant of *I. fumosorosea* and the possibility of the presence of biologically active secondary metabolite compounds in the biosynthesis of Ag NPs.

Material and Methods

Preparation of crude Fungal Filtrate

Isolation of *I. fumosorosea* was obtained from the Iraqi Ministry of Agriculture-Plant Protection Research Center. Fungal isolate was cultured on media potato dextrose agar at 26 ± 2°C for 15 days.. Were taken Two discs (7 mm diameter) from fungal media of *I. fumosorosea* and placed in a sterile flask containing 500 mL of potato dextrose broth, and incubated at 28°C for 14 day. After the incubation, the mycelia was separated by filtration. The filtrate was sterilized by using Millipore 0.45 um syringe filter . Keep the filtrate in the refrigerator until use (8).

Gas chromatography - Mass spectrometry (GC-Mass) analysis

GC-MS analysis of the crude fungal filtrate of *I. fumosorosea* was performed by instrument of gas chromatography–mass spectrometry Shimadzu's in the Science and Technology Center- Baghdad. Retention time and mass spectrometry were used to identify the secondary metabolism components by comparing the mass spectrum of unknown peaks to those kept in the National Institute of Standards and Technology (NIST) collection.

Synthesis of Ag NPs nanoparticles

Silver nanoparticles Ag NPs were prepared by mixing 100 ml of *I. fumosorosea* crude filtrate with 900 ml of 50 mM silver nitrate solution by using a shaker at 150 rpm for three days in the dark. A visual inspection of the solution (color shift from yellow to brown), indicated the biological reduction process and creation of silver nanoparticles. The solution of nanoparticles was centrifuged for half an hour at a speed of 4000 rpm to purify and produce silver nanoparticles as powder. The deionized water was added to the powder of silver nanoparticles for washing, then dried by a glass Petri plate, for five hours at 55 °C.(16).

Biosynthesis and characterization of AgNPs

Shimadzu UV-Vis Spectrophotometer was used to determine the successful synthesis of AgNPs. Using FTIR, different functional groups involved in the stabilization and capping of the produced nanoparticles were characterized. Typical peaks were seen in the 400–4000 cm-1 range. Surface morphological analyses were conducted by using the FESEM technology for the evaluation of the size and shape of created AgNPs.

Results and Discussion

The presence of 15 chemical compounds as secondary metabolites was discovered in the crude filtrate of *I. fumosorosea* according to Figure 1 and Table 1 using GC-Mass analysis based on the retention time of the separation column where some compounds such as acetic acid, N-Methoxy-N-methylacetamide, dimethyl (bis [(2Z) -pent-2-en-1-yl), sorbitol, hexanedioic acid, eicosane and 9-octadecenoic acid, (E) were recorded, reflecting the ability of the fungus to produce secondary metabolites in growth media.

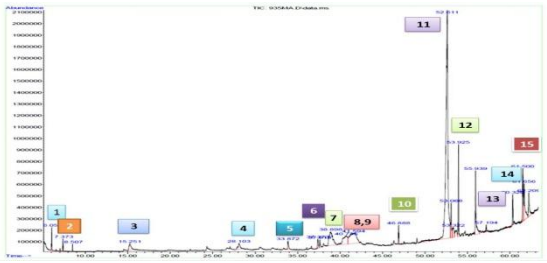

Figure 1. GC-Mass chromatogram of crude filtrate of *I. fumosorosea*

Table 1. GC-Mass spectral analysis of crude filtrate of *I. fumosorosea*

No.	Name of compound	retention time/ min	peak area %
1	Acetic acid, propyl ester	6.053	0.83
2	Isobutyl acetate	7.373	0.42
3	N-Methoxy-N-methylacetamide	15.249	1.40
4	1 2(1H)-Quinolinone, hydrazone	28.103	1.30
5	1 DL-Proline, 5-oxo-, methyl ester	33.870	0.78
6	Dimethyl(bis[(2Z) -pent-2-en-1-yl	37.373	0.72
7	2-Allyloxy-N-methoxy-N-methyl-ac	38.899	5.42
8	Sorbitol	40.734	4.20
9	L-Arabinitol	41.591	7.84
10	Heptadecane	46.889	0.84
11	Hexanedioic acid, bis(2-ethylhex	52.610	48.80
12	Z-5-Nonadecene	53.067	2.27
13	Eicosane	57.194	0.45
14	Heneicosane	60.320	1.28

15	9-Octadecenoic acid, (E)-	61.497	3.83

Characterization of AgNPs

The absorption peak in the UV spectra in Figure 2 shows that AgNPs were synthesized using 1 millimolar silver nitrate with crude filtrate of *I. fumosorosea* where a change in color was observed in the solution to brown. It has been reported in previous studies that the Absorption of silver nanoparticles was recorded between 300-700 nm, Therefore, the presence of silver nanoparticles was confirmed in the current study with peak absorption at 419 nm. The peak height value was consistent with (3) when using the endophytic fungal extract in the biosynthesis of silver nanoparticles.

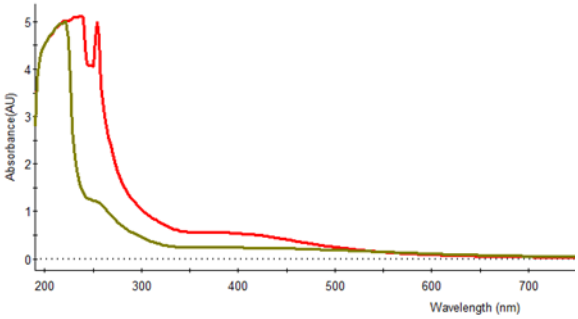

Figure 2. UV-Vis of Biosynthesis Ag NPs using crude filtrate of *I. fumosorosea*

FT-IR spectral analysis in the range of 450- 4000 cm-1 was used to record the multiple functional groups in the crude filtrate of *I. fumosorosea* shown in Figure 3, which acts as stabilizing, capping and reducing agents in the biosynthesis of Ag NPs. The FT-IR bands of *I. fumosorosea* crude filtrate indicate triterpenoids, flavonoids, furanoids, sugars, coumarins, quinones, tannins, phenols and acids. The strong peak at 3437.83 cm-1 indicates amine groups and N-H stretching bending. Also, the peak at 2329.33 cm-1 indicates the presence of symmetric CH3 stretching. The strong peak at 1638.17 cm-1 revealed a tertiary amide (C-O stretching), in addition to the peak at 1383.38 cm-1 revealed alkane formation (C-H stretching). The peaks at 822.61 and 694.84 cm-1 are assigned to halogen compounds. These analytical results are consistent with previous results (15).

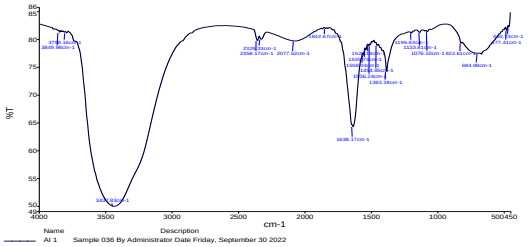

Figure 3. FTIR spectra of silver nanoparticles produced by crude filtrate of *I. fumosorosea*

Using a variable magnification of up to 200 nanometers, the field emission scanning electron microscope (FESEM) was used to distinguish the size and shape of the composite nanoparticles from silver nanoparticles by crude filtrate of *I. fumosorosea*. The particle shape was semi-spherical and the diameter of Ag NPs was found within 44.66-89.23 nm in Figure 5. Our results were consistent with those obtained in (1).

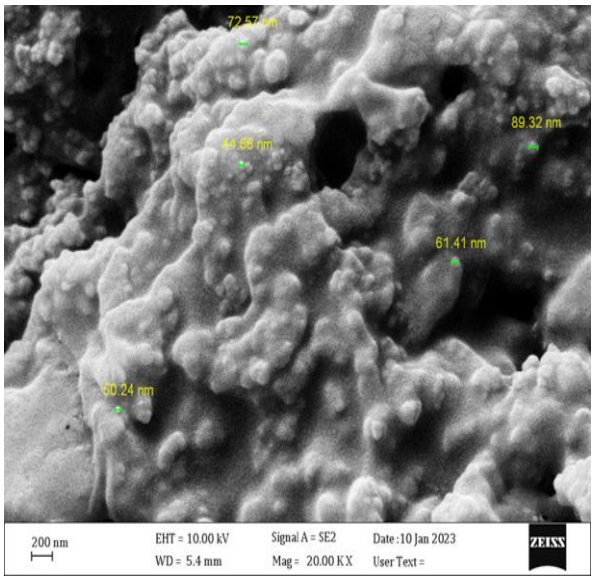

Figure 4. FESEM of silver nanoparticles synthesised by crude filtrate of I. fumosorosea

Conclusion

There has been a recent acceleration in nanoscience, with a shift in the synthesis of nanoparticles from chemical and physical methods to biological methods based on plant extracts and fungal secondary metabolites, due to their environmental safety, accessibility, and cheaper price. Entomopathogenic fungi are an important factor in insect control and this study came for the purpose of introducing and benefiting from them in the biosynthesis of nanoparticles as reducing agents for metals in the synthesis process due to their containment of secondary chemical compounds, which can be diagnosed and confirmed using GC-Mass and FTIR techniques. This study came to open the door for a new use of insect pathogens in a new field, which is nanotechnology, in order to produce a new generation of insecticides that can be more effective in controlling insect pests.

Conflict of interest

The authors declare no conflict of interest.

References

1- Aina, A.D.; Owolo, O.; Adeoye-Isijola, M.; Amodu, S.; Olukanni, O.D.; Aina, F.O.; Lateef, A., and Adediji, I. 2018. Mycosynthesis of silver nanoparticles using Lentinus squarrosulus and its antibacterial activities. Science Focus 23 (2): 44-54.

2- Albehadli, K. and Mohmed, A. S.2022. Laboratory evaluation of the aphidicidal activity of some entomopathogenic fungal culture filtrate against aphid *Schizaphis graminum*. AIP Conference Proceedings 2398(1):040020 DOI: 10.1063/5.0093653.

3- Govindappa, M.; Farheen, H.; Chandrappa, C. P.; Channabasava, R. R. V. and Raghavendra, V. B. 2016. Mycosynthesis of silver nanoparticles using extract of endophytic fungi, *Penicillium* species of *Glycosmis mauritiana*, and its antioxidant, antimicrobial, anti-inflammatory and tyrokinase inhibitory activity, Advances in Natural Sciences: Nanoscience and Nanotechnology, 1-9, doi:10.1088/2043-6262/7/3/035014.

4- Hilal, S. M.; Mohmed, A. S.; Barry, N. M.; and Ibrahim, M. H. 2021. Entomotoxicity of TiO2 and ZnO Nanoparticles against Adults *Tribolium*

castaneum (Herbest)(Coleoptera: Tenebrionidae)," in IOP Conference Series: Earth and Environmental Science (IOP Publishing Bristol, UK), 012088. doi:10.1088/1755-1315/910/1/012088.

5- Jabbar, A Sh. ; Mohmed, A. S. and Hussein, A. M. 2023. Mycosynthesis of Silver Nanoparticles and Evaluation as Insecticidal Against the Sunn Pest *Eurygaster testudinaria* in Vitro IOP Conf. Series: Earth and Environmental Science 1158 doi:10.1088/1755-1315/1158/7/072013.

6- Lee, S.H. and Jun, B.H. 2019. Silver nanoparticles: synthesis and application for nanomedicine. Int J Mol Sci.; 20(4):1-23.

7- Mohmed, A. S.2019a. In vitro efficacy of leaf extract *of Iresine herbstii* and *Isaria fumosorosea* to control aphis gossypii glover. Plant Archives 19, Supplement 2, pp. 1224-1228.

8- Mohmed, A.S. 2019b. Toxicity of Two Entomopathogenic Fungi against Black Bean Aphid *Aphis fabae* Scopoli under Laboratory Conditions. Biopestic. Int., 15(2): 151-154.

9- Mohmed, A. S. 2020. Insecticidal Potential of Tio2 Nano-Particles Against *Eurygaster Testudinaria* (Geoffroy) Under Laboratory Conditions. Int. J. Agricult. Stat. Sci., 16(1):1221-1224.

10- Reverberi, A. P.; Kuznetsov, N. T.; Meshalkin, V. P.; Salerno, M. and Fabiano, B. 2016. Systematical analysis of chemicalmethods in metal nanoparticles synthesis Theor. Found. Chem. Eng. 50 59– 66. https://doi.org/10.1134/S0040579516010127**.**

11- Roy, S. and Das, T. K.2015. A Review on Biosynthesis of Silver Nanoparticles Using *Aspergillus* Species. Advanced Science, Engineering and Medicine . 7, 1–10.

12- Santos, T.S.; Silva, T.M.; Cardoso, J.C.; Albuquerque-Júnior, R.L.C.d.; Zielinska, A.; Souto, E.B.; Severino, P.; Mendonça, M.d.C. 2021. Biosynthesis of Silver Nanoparticles Mediated by Entomopathogenic Fungi: Antimicrobial Resistance, Nanopesticides, and Toxicity. Antibiotics , 10, 852. https://doi. org/10.3390/antibiotics10070852.

13- Singh, D.; Raina, T.K. and Singh, J. 2017. Entomopathogenic fungi: An effective biocontrol agent for management of insect populations naturally, Journal of Pharmaceutical Sciences and Research, 9 (6) : 830–839.

14- Taha, Z. K.; Howar, S. N.and Sulaiman, G. M. 2019. Isolation and Identification of *Penicillium italicum* from Iraqi Citrus Lemon Fruits and its Ability Manufacture of Silver Nanoparticles and their Antibacterial and Antifungal activity. Research J. Pharm. and Tech. 12(3):1320-1326.

15- Taware, P. V.; Chautmal, P. G.; Waghmare, A. D.; Waghmare, S. A.; Kate, O. R. K. and Deepika, A. P.2020. Mycosynthesis of silver nanoparticles from mycelium extract of *Penicillium* species and its antimicrobial activity against clinical isolates. J. of Global Biosciences. 9(8), : 7808-7825.

16- Tomah, A.A.; Alamer, I.S.A.; Li, B. and Zhang, J.Z. 2020. Mycosynthesis of Silver Nanoparticles Using Screened Trichoderma Isolates and Their Antifungal Activity against *Sclerotinia sclerotiorum*.

Nanomaterials (Basel).;10(10):1955. doi: 10.3390/nano10101955. PMID: 33008115; PMCID: PMC7599925.

17- Yao, W.; Qu, M.; Cui, X.; Xia, R.; Liu, X.2020. Optimization of synthesizing silver nanoparticles from *Trichoderma* strains for inhibition of *Fusarium oxysporum*. Sheng Wu Gong Cheng Xue Bao. 36(9):1859-1868. Chinese. doi: 10.13345/j.cjb.200027. PMID: 33164462.