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Production of Biosynthesized Silver Nanoparticles using *Metarhizium anisopliae* fungus for the Treatment of Petroleum Pollutants in Water

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Abstract

In this study, a silver nanoparticle (AgNPs) was created using a biological technique from an extract of the fungus *Metarhizium anisopliae*. The characteristics of the prepared AgNPs were identified by utilizing the optical, ultraviolet, and infrared absorbance spectroscopy. The shape, size, and charge distribution on the particles were determined by using scanning electron microscopy and zeta voltage analysis .The analysis of biological activity of the silver nanoparticles showed its effectiveness in treating pollutants, as confirmed by the reduction of higher than 93% weight of crude oil in contaminated water samples. The crude oil mass was effectively transformed into the gelatinous mass that lacks consistency and emulsification. The chemical analysis of NP-treated and untreated crude oil-contaminated water samples was performed using gas chromatography mass spectrometry (GC MASS). The results displayed the emergence of 55 graphic peaks, each of them indicating a chemical compound, in the control sample, while in the study sample, about 51 of these peaks disappeared and the area of the remaining 4 peaks was reduced. The silver nanoparticles' capability to maintain their effectiveness under cryogenic storage conditions for six months was tested and compared to that of the fungal isolation before the production of the silver nanoparticles. The results showed no significant changes in the shape, size, and efficiency of the silver nanoparticles in the treatment of oil pollutants in water. The results indicated the higher efficiency of the silver nanoparticles, as compared to chemicals, in treating petroleum pollutants as well as enhancing the solubility, emulsification, and degradation of hydrocarbons . In addition, the AgNPs are characterized by the availability of inexpensive, easy, fast to produce, and environmentally friendly production materials, as compared to the usage of chemical products that are highly toxic to aquatic organisms, expensive to produce, and highly accumulative in the ecosystem, i.e. environmentally unsafe.

Keywords: AgNPs, Biodegradation, Biotechnology, *Metarhizium anisopliae*, Petroleum Pollutants.

Introduction

The issues related to environmental pollution have begun to appear since the discovery and consumption of crude oil, becoming a threat to human life. With the expansion in the production and consumption of

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Baghdad Science Journal

oil, increased risks of environmental pollution started to manifest, reaching higher risks of the spread of oil spills and various oil residues to water surfaces. Oil is one of the most dangerous types of pollutants, because of the high quantities of its derivatives as well as its toxic substances that exert remarkably harmful impacts on humans and animals. The toxicity of oil is due to its composition of many hydrocarbons, such as benzene, which is a carcinogenic hydrocarbon. In addition, oil contains ether and gasoline, which are dangerous compounds when available in high concentrations. Moreover, phenolic compounds contained in the oil are, upon inhalation, among the causative agents of allergy [1]. Collectively, these impacts can lead to the death of aquatic organisms. Furthermore, oil, especially crude oil, contains hydrogen sulfide and other toxic gases that are emitted when hydrocarbon particles evaporate or dissolve [2]. Biodegradation is considered one of the most important and safe mechanisms to eliminate oil pollutants from water[3]. This process is achieved by microorganisms, e.g. bacteria, fungi, and algae, through their consumption of hydrocarbons present in the oil. The degree of consumption depends on the nature of the microorganism [4]. The process of biological treatment is accomplished through the decomposition of oil hydrocarbon compounds by microorganisms and their conversion into simple and environmentally friendly compounds, such as carbon, carbon dioxide, nitrogen, phosphorous, and others [5]. Fungal species, e.g. *Aspergillus*, have recently been utilized in the field of biological treatment and disposal of environmental pollutants resulting from oil waste. It was found that these species tend to consume hydrocarbons present in petroleum products [6].

Nanotechnology technique deals with particles ranging from 1 to 100 nm [7]. Nanomaterials differ from their larger counterparts in terms of their physical, chemical, and structural characteristics, the nanomaterials' extraordinary magnetic, electrical, and optical characteristics and surface activity are due to their nanoscale shape and size [8].

There are many studies in field of biosynthesis of nanoparticles using different biological materials, like study of Shah et al, silver nanoparticles (AgNPs) were synthesized by using *Silybum marianum* plant extract [9] and Abd-Elhady et al, used biological approach to synthesis Extracellular Silver Nanoparticles from *Streptomyces aizuneusis* [10].

At the present time, new innovative methods have been introduced to control and reduce environmental pollution, including nanotechnology which improves the properties of fungi at low costs [11]. The present study aims at synthesizing and diagnosing silver nanoparticles produced from the extract of the fungus *Metarhizium anisopliae*. The effectiveness of these NPs in treating oil pollutants in water, as well as their storage potential, were determined.

Materials and Methods

Fungal Isolate

A local fungal isolate, identified as *Metarhizium anisopliae*, was grown in Potato Dextrose Agar-PDA culture medium sterilized with an autoclave for 15 minutes. Two grams of tetracycline were added per liter of medium to prevent bacterial growth. The culture dishes were incubated in an incubator (Memmert) at 26±2 ˚C and a relative humidity of 75±5 % for a week, until a complete fungal growth was achieved, according to the method described in [12].

Crude Oil Sample

Oil samples were obtained from Kirkuk oil fields according to the following specifications:

The American Petroleum Institute (API) gravity at 60 Fº	34.1
Density at 59 Fº	0.8540
Salt content (%)	0.0012
Water and sediment content (Vol %)	0.05

Preparation of Fungal Suspension:

The fungal suspension was prepared by pouring 5 ml of sterile distilled water to a petri dish containing the

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Baghdad Science Journal

fungal colonies upon their complete growth on the culture medium. The spores were separated by an L-shaped harvester. The fungal suspension was filtered through a Whatman grade 1 filter paper after adding another 5 ml of distilled water to the sides of the filter paper to eliminate the mycelium and obtain the fungal cells (Fungal stock suspension), based on the method of [13].

Cell Number Counting in the Fungal Suspension:

Following the method of [14], the counting chamber of a Neubauer hemocytometer was utilized to count the number of cells. One drop (0.1 ml) of the stock fungal suspension was placed on the slide, which was covered with a cover slip prior to counting the number of cells in the four squares of the chamber. An optical microscope was used to examine the slide at 40 x power. The calculations were made according to the following equation:

Number of spores = (N / 80) *10^6 * 10

where:

N = the average number of cells counted in the four squares.

80 = sum of the minute squares within the four counted squares.

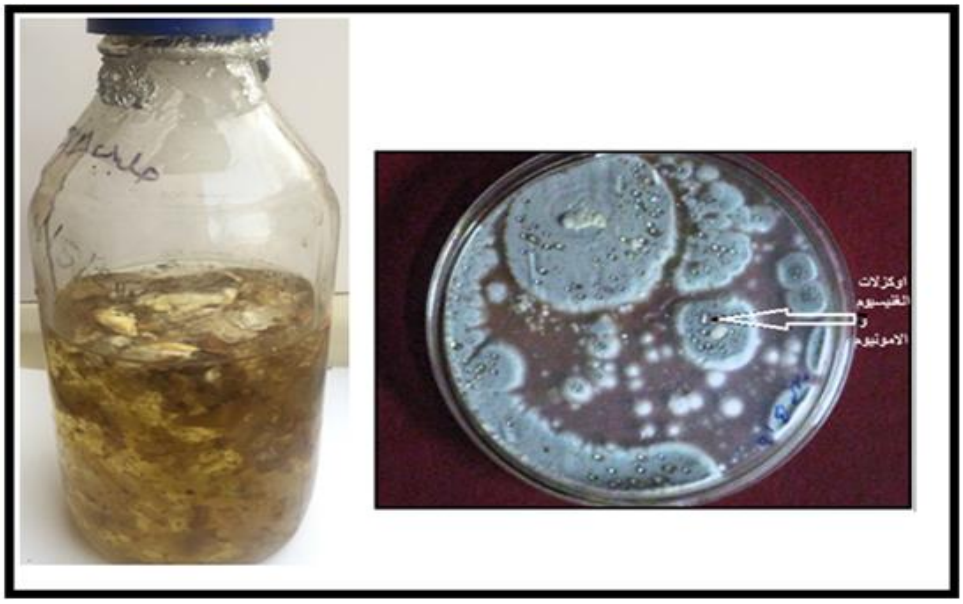
10^6 = concentration correction factor.

10 = volume correction factor.

The Effectiveness of the Silver Nanoparticles

Preparation of *Metarhizium anisopliae* extract:

To obtain its biomass, the fungus isolate was grown in a petri dish containing sterile potato agar and dextrose medium. The culture was incubated at 26 ± 2 ˚C and relative humidity of 75 ± 5 % for 7 days. Four discs of the grown colonies were collected, with a diameter of 5 mm, and inoculated within a sterile liquid dextrose potato broth (PDB) medium. Two grams of tetracycline per liter of medium were added, followed by the incubation of the flasks in a shaker incubator (100 cycles per minute) at 26 ± 2 ˚C for 21 days under wet conditions. Fig. 1 shows the preparation of the biomass of *Metarhizium anisopliae*.



(A) **(B)**

Figure 1. Preparation of the biomass of *Metarhizium anisopliae*; (A) Fungal biomass (x1), (B) Fungal colonies grown on PDA culture medium (x1).

The fungal biomass was harvested after 21 days of growing using a sterile filter unit (Buchner funnel and sterile Whatman No.1 filter paper) under vacuum pressure and aseptic filtration conditions. The

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harvest was washed with distilled water three times, followed by a washing step with deionized distilled water. Cell breakage technique with ultrasound for 5 minutes and 30 seconds intervals in one run was applied to break down the fungal cells. The harvest was filtered using 0.45 µm Millipore filters. The filtrate was kept at 26 ± 2 ˚C and relative humidity of 75 ± 5 % until use, according to the method described in [15].

Preparation of the Silver Nanoparticles

The aqueous solution of silver nitrate at a concentration of 50 mmol was prepared as follows:

molecular weight of $AgNO_3$= atomic weight of Ag (107.87gm/mole) + atomic weight of N (14 gm/mole) + atomic weight of O (16 gm/mole) x 3 = 169.87 gm/mole.

Therefore, 1000 ml of a 50 mmol silver nitrate solution contains: (169.87 x 50) / 1000 g = 8.4935 gm of silver nitrate. It was added to 1000 ml of deionized water to obtain a 50 mmol solution of silver nitrate.

The solution was heated to 60 ° C for 10 minutes with stirring, using a water bath and ultrasound waves. The silver nanoparticle was prepared by distilling 50 ml of fungal extract into 950 ml of silver nitrate solution in a vibrating incubator at 60 ° C for 120 hours. The color changes were monitored, as evidenced by the formation of silver nanoparticles via the biological reduction process, according to the method of [16].

Diagnosis of Silver Nanoparticles

UV-VIS Spectrophotometry

The optical characteristics of the nanoparticle, silver nitrate, and fungal biomass extract, were determined separately by 10 times dilution of 2 ml of the prepared solutions with deionized water to reduce the false readings. The samples were transferred for examination by spectrophotometry and ultraviolet waves, with the appropriate wavelength being fixed within a range of 190 to 850 nm [17].

Fourier Transform Infrared (FTIR) Spectroscopy

This method was utilized to determine the active groups of both the fungal biomass extract alone and its mixture with silver nitrate solution (silver nanoparticles). These active groups function as encapsulating and reducing agents for silver nanoparticles. The samples were prepared by depositing a drop of each sample on a glass slide and drying it at 60 ° C in an oven for 30 minutes. The product was made into a paste using a high-viscosity liquid substance (Nujol paraffin oil). A small amount of the paste was placed between two discs of potassium bromide to form a very thin layer, which was examined with a Fourier transform infrared device [18].

Scanning Electron Microscopy (SEM)

The structural characteristics of the samples were determined in terms of size and shape. The samples were prepared by mounting drops of the silver nanoparticles solution separately on glass slides, followed by drying at 60 ° C for 30 minutes and staining with crystal violet for one minute. The specimens were washed with distilled water and fixed with Giemsa stain for one minute. They were then coated with gold in an atmosphere saturated with argon gas under pressure of 50 Pa and a current of 50 mA for 50 seconds. The specimens were examined with an SEM at 30000 X magnification [19].

Zeta Potential

The stability of silver nanoparticles was evaluated by using a zeta potential device with a measurement range of -160 to +160 mV [20].

Storability of the Nanoparticle

A sample of the silver nanoparticles was placed in clean and sterilized containers and stored at 4 ° C. Six months after production, the effectiveness of the stored nanoparticle was tested in terms of causing phenotypic changes, emulsification, and disintegration of crude oil. This was performed by incubating a mixture of crude oil-contaminated water samples with the nanoparticle for one week and comparing the results with those of the control sample (crude oil-contaminated water without nanoparticle).

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Baghdad Science Journal

Estimation of Quantitative Loss Percentage of the Crude Oil

Non-specialized Chemical Tests

The bio-nanoparticle was chemically tested for its efficiency in breaking down crude oil. The silver nanoparticles were added to 50 ml of sterile distilled water, and then 1 ml of the crude oil was added in 250 ml conical flasks. The mixture was placed in a shaking incubator at 30 ° C at a 50 rpm for 14 days, with the process being repeated twice. The mixture was placed in special tubes and centrifuged at 10,000 rpm for 20 minutes to remove impurities. The supernatant was combined with hexane extract and a few drops of hydrochloric acid were added until the pH of the medium reached 2. The hydrocarbons were extracted by adding the solvent chloromethane, after placing the sample in a separation funnel, to separate the water layer from the hydrocarbon layer which is dissolved in the solvent. The last layer was filtered through a filter paper containing anhydrous sodium sulfate (Na_2SO_4) as a desiccant agent and utilizing a clean beaker to collect the filtrate. The solvent was evaporated by using a rotary evaporator at room temperature, and the sample was weighed to calculate the weight of the remaining hydrocarbons. The method was repeated using the control sample. The rate of degradation was determined according to the method of [21]. through the equation below:
Crude oil degradation rate = Weight of remaining crude oil / Weight of control crude oil *100.

Detection of Crude Oil Decomposition Using Gas-Mass Chromatography

A volume of 1 ml of crude oil was added to 50 ml of sterile distilled water in a 200 ml conical flask. After stabilization, the nanoparticle was added to the mixture in two replicates. In addition, the negative control (water with crude oil without nanoparticles) and positive control (water with nanoparticles without crude oil) samples were prepared. The flasks were placed in a shaking incubator at 30 ° C at a rotation speed of 100 rpm for 14 days. They were then placed in special tubes in a centrifuge for half an hour at a rotation speed of 12000 rpm to precipitate the impurities.

The filtrate was dissolved by adding hexane solvent in the ratio of 1: 1 volume in the separation flasks, that were shaken for a while and left in a vertical position until the separation process was completed when the hexane layer was collected. The samples were concentrated with a rotary evaporator at a temperature of 40 ° C. The samples, which now represent the remainder of the hydrocarbons, were tested for the level of dissociation and decomposition of crude oil components by the nanoparticle. For this analysis, a Gas-Chromatography device (Shimadzu Model 2014) was used, with a 30 m long capillary column of the CPSIL5-CB type, into which 0.4 mL of each sample was injected. The conditions used to operate the device involved nitrogen as carrier gas at a flow rate of 25 milliliters / minute, while the FID detector temperature was set at 270 ° C.

The separation column was prepared with an initial temperature of 80 ° C and programmed to raise the temperature at a rate of 5 ° C / minute to a temperature of 260 ° C, until it reaches the end of the total time of 60 minutes, the peaks of the used compounds were compared with the peaks of standard materials that appear at the same retention time. The area was calculated to estimate the amount of hydrocarbons remaining after silver nanoparticles decomposition according to the following equations[22]:

Area = 1/2 base * height.

Percentage of residual hydrocarbons = sample area / total area * 100

Results and Discussion

Characteristics of the Prepared Silver Nanoparticles

UV-VIS Spectroscopy

The optical and spectroscopic characteristics of the prepared silver nanoparticles were studied. The presence of color changes was observed when the biomass extract of *M. anisopliae* was added to silver nitrate solution and compared to the solution containing the fungus biomass extract without silver nitrate $AgNO_3$, which did not demonstrate any color changes. These colors ranged from light brown to

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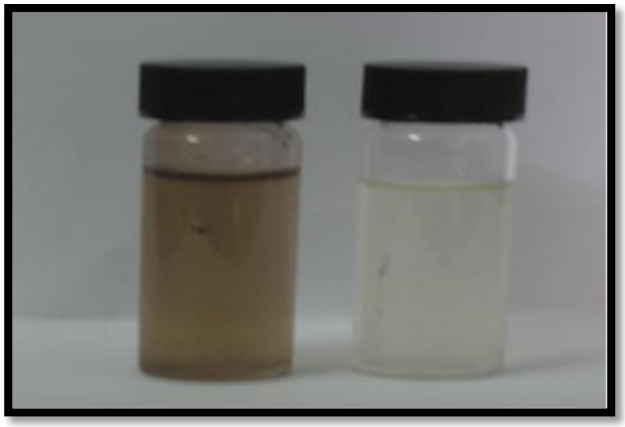
Baghdad Science Journal

dark brown, as displayed in Fig. 2. It was also observed that the color change process was stabilized after 120 hours at a temperature of 26 ± 2 ° C.



(A) (B)

Figure 2. Color changes that appeared when preparing the nanoparticle using *M. anisopliae;*
A) colloidal solution of silver nanoparticles with fungal biomass extract.
B) Fungal biomass extract without silver nitrate.

The occurrence of these changes is evidence of the formation of silver nanoparticles while showing of this particular color is due to the excitation of the surface plasmon of the particles formed, i.e. surface plasmon resonance (SPR) [23,24]. This property appears on the surfaces of some metals as a result of the collective movement of free electrons in the nanoparticle when light falls on them. The direction of electron movement changes over time with the same oscillation of the incident optical electromagnetic wave. This characteristic is clearly found in silver, gold, and copper, provided that the nanomaterial is colloidal, in the visible light region. It is responsible for changing the colors of these elements when they reach the nanoscale and has many properties and applications. This characteristic is based on the particle size, shape, and medium in which the particles are present [25].

The above-mentioned results agree with those reported by [26], during his study of the effects of silver nanoparticles prepared by biological methods. He recorded the presence of color changes, upon adding the biomass extract of *B. Bassiana* to a silver nitrate solution, within 120 hours at a temperature of 25 ° C. These color changes also appeared when preparing silver nanoparticles using the fungi species of *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N, and Fusarium oxysporum [27,28]. The color changes occur within 5 days of the reaction and remain constant for 60 days.

Ultraviolet spectroscopy was used to measure the surface plasmon excitation of the silver nanoparticles in solution, by recording the highest absorbance at the wavelength of 293.68 nm for the silver nanoparticles prepared by the extract of *M. anisopliae* as illustrated in Figs. 3, 4, and 5. The ultraviolet spectrum of silver nanoparticles produced by *Penicillium citrinum* at a wavelength of 265 nm was reported to indicate the presence of tyrosine and tryptophan residues, thus confirming that the proteins secreted by the fungus are important for the formation and stability of the silver nanoparticles [29]. The fungus *Aspergillus ochraceus* recorded the highest absorbance at the wavelength of 280 nm. This indicates that the fungus released its enzymes into the fungal filtrate [30]. The highest absorbance at the wavelength of 260 -290 nm is due to the presence of amino acids and phenylalanine, along with tyrosine and tryptophan present in the protein. This is evidence of the release of proteins and enzymes by the fungus to the aqueous medium, which has led to the suggestion of the mechanism by which silver ions are reduced, by [31].

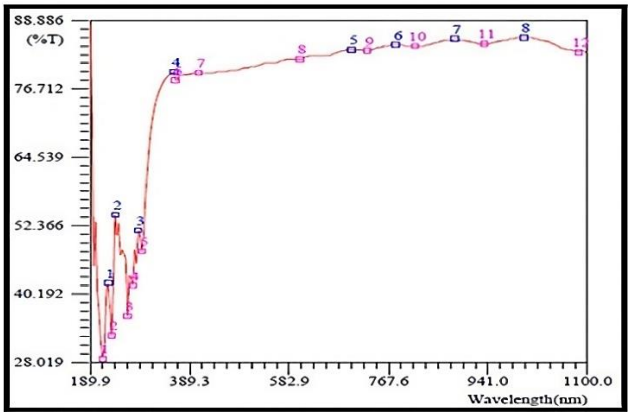


Figure 3. Absorption spectrum of *M. anisopliae* tested by using UV-VIS spectrophotometer.

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Baghdad Science Journal

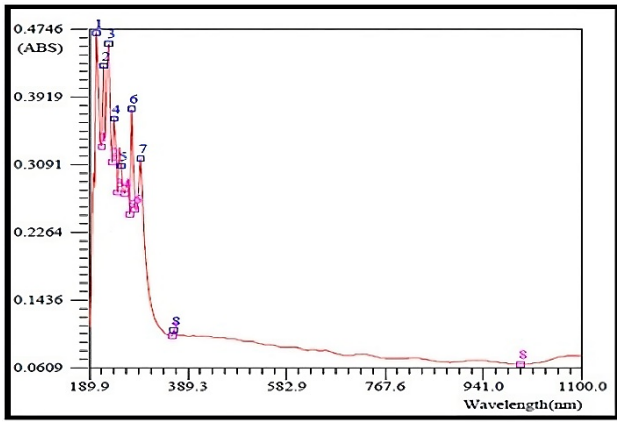

Figure 4. Absorption spectrum of silver nitrates solution tested by using UV-VIS Spectrophotometer

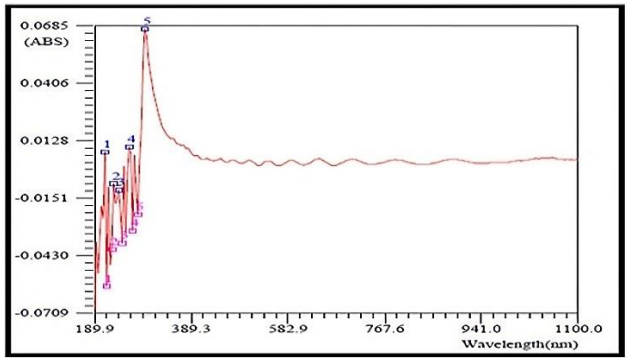

Figure 5. The absorption spectrum of silver nanoparticles tested by using UV-VIS spectrophotometer.

The above-mentioned studies reflect the high activity of the biomass extract of *M. anisopliae* in reducing substances by the presence of fungal enzymes, including anthraquinones, naphthoquinones, and nitrate reductase, that enhance the reduction of nitrates to nitrites and ammonia [32]. Fungi are characterized by their ability to assist the formation of larger quantities of silver nanoparticles, as compared to bacteria, due to their ability to secrete a large amount of enzymes that reduce silver into metal particles and form silver nanoparticles [33].

FTIR Spectroscopy

Infrared spectroscopy is a strong tool for identifying the kinds of chemical bonds in a molecule, by producing an infrared absorption spectrum that is similar to the molecular "fingerprint". A number of power unit peaks were obtained when analyzing *M. anisopliae* biomass extract, silver nitrate solution, as well as the prepared silver nanoparticles, as shown in Figs. 6, 7, and 8. Infrared spectroscopy demonstrated the presence of amide bonds between amide bands, through the values in the regions of 1200 to 1800 1 / cm and the presence of the Alkyl halides group (C-l), through the values in the areas 505.35, 576.72, 794.67 and 842.89 1 / cm. Carboxyl (COO-H) aggregates were recorded in regions 939.33 and 1039.63 1 / cm. The analysis also showed the presence of aromatic compounds in regions 746.45, 867.97, and 889.18 1 / cm. Also, regions of ketones (C-C) and aldehydes (C = O) appeared in regions 1139.63 and 1708.93 1 / cm. As for the presence of ethers and esters, they were indicated in the area 1192.01 1 / cm, in addition to the presence of energy regions of alkanes at 817–1689.64 1 / cm and alkanes at 1377.17, 1467.83, and 2804.45 1 / cm. Alcohols were found at the energy regions 1211.3, 1236.37, and 1257.59 1 / cm. Spectroscopy also recorded the existence of a group of C = O and a group of N-H, represented by the values in the regions of 1400-17001 / cm. As for the energy regions of amines, they were located at 1639.49-1514.12 1 / cm, indicating the presence of protein on the surface of the Ag particle [34]. The area at 1014.56-1303.81 indicated the presence of C-O in the active groups of esters, ethers, and alcohols, distinguished by their strong ability to bond with silver ions [31,35].

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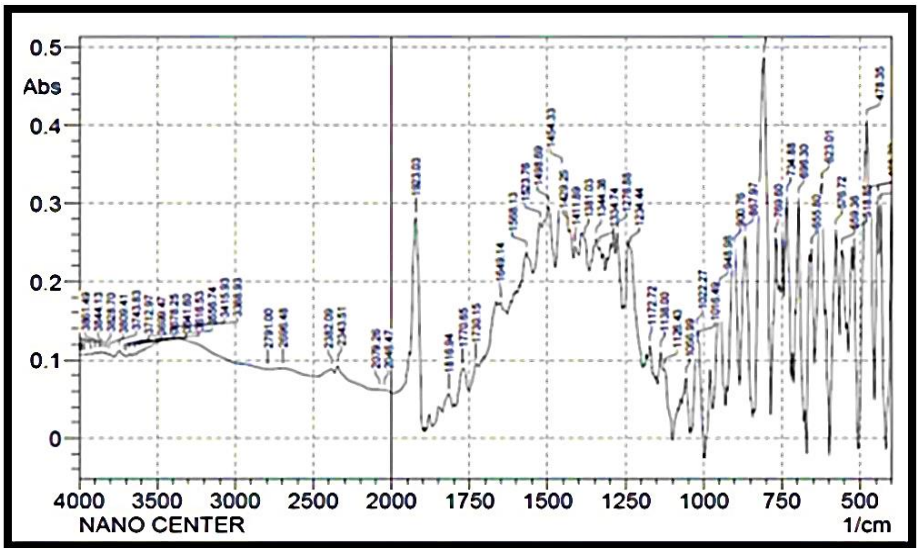

Figure 6. Fourier transform infrared spectroscopy of *M. anisopliae* extract

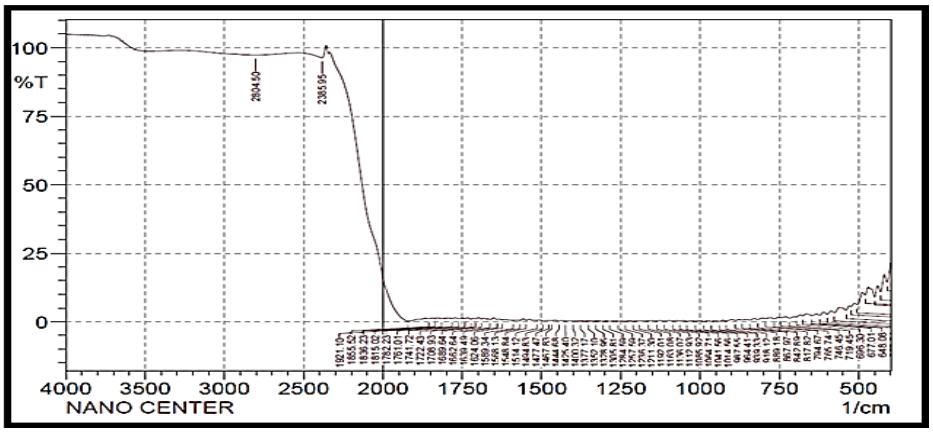

Figure 7. Fourier transform infrared spectroscopy of silver nitrate solution.

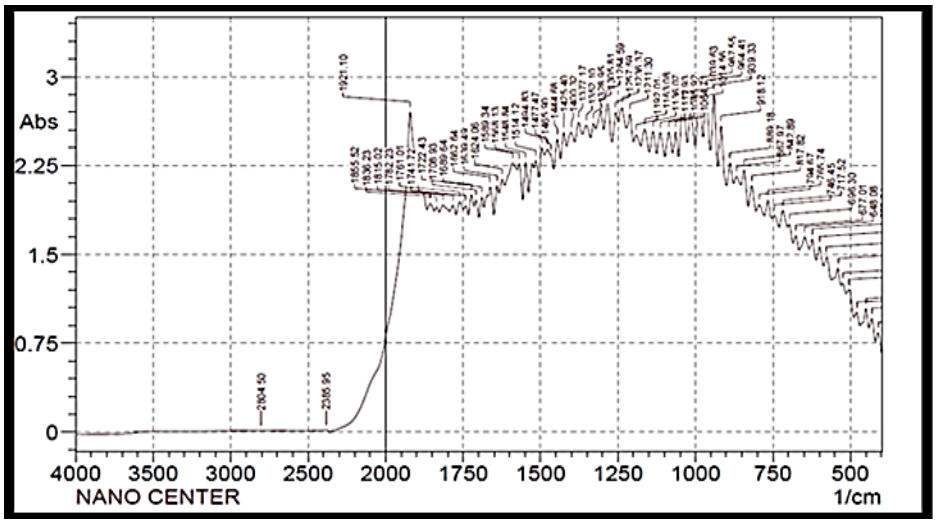

Figure 8. Infrared spectroscopy of the silver nanoparticles prepared with *M. anisopliae* aqueous extract

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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

The energy units of the carboxyl and Alkyl halides groups reflect the presence of proteins, which is an indication of their important role in the biological reduction of the silver nitrate and the production of the silver nanoparticles, as well as their functions as stabilizers that envelope and prevent silver nanoparticles from aggregating. The regions of ketones, ethers, esters, and aromatics have a similar role [33]. Thus, *M. anisopliae* extract is characterized by being rich in active compounds that contribute to the biological reduction of the silver nitrate and the production of silver nanoparticles. Being a rich source of capping agents and stabilizing silver nanoparticles, this extract competes with chemical and physical methods that use toxic, harmful, and costly substances [36].

Soleimani et al, [37] indicated that the aqueous extract of *B. bassiana* contains capping, stabilizing, and reducing agents, represented by alcoholic, protein, and phenolic groups. These groups are among the most active groups that participated in the biological reduction process, as indicated by the infrared spectroscopy values.

Examination by Scanning Electron Microscopy

The size and shape of the prepared silver nanoparticles were determined by electron microscopy. Image 1 shows that the silver nanoparticles prepared with the extract of *M. anisopliae* were characterized by spherical and oval shapes, while the size range was 34.7 to 141.6 nm, with an average of 76.65 nm.

The shape and size of the silver nanoparticles play a significant role in determining their physical, chemical, and biological properties. [38], found the size of the silver nanoparticles prepared by the aqueous extract of *Beauveria bassiana* to be in the range of 10–50 nm. The spherical shape of the silver nanoparticles produced from the aqueous extract of *Penicillium citrinum* appeared when examined under an electron microscope[29].

Image 1. A scanning electron microscope image showing the shape and size of the nanoparticle prepared with *M. anisopliae* extract; A = 98.2 nm, B = 65.11 nm, C = 35.31 nm.

Zeta Potential Analysis

The zeta potential is usually referred to using the Greek letter zeta (ζ). The value of zeta potential of silver nanoparticles is -75.65 mV for silver nanoparticles as shown in Fig. 9. It is an important indicator of the stability of the nanoscale solution. The zeta potential size refers to the degree of electrostatic repulsion between adjacent, similarly charged particles in the dispersion. For sufficiently small particles, the high zeta potential value will provide stability, that is, the dispersion will resist aggregation. Therefore, colloids with high zeta potentials (positive or negative) are electrically

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stabilized, while colloids with lower zeta potentials tend to clump and agglomerate, i.e. the higher the zeta potential the more stable the substance is[39] (Scheme 7).

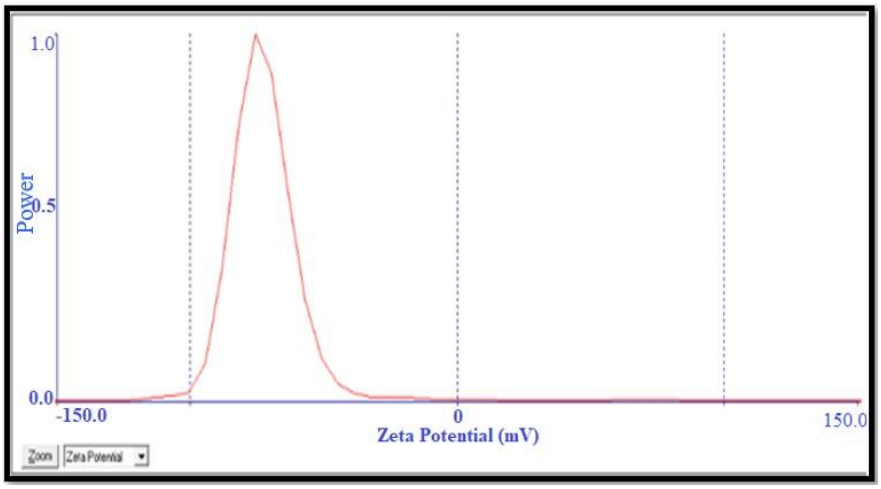

Figure 9. Zeta potential analysis of silver nanoparticles prepared with *M. anisopliae* extract.

Detecting the Decomposition of the Crude Oil Using Non-specialized Chemical Methods

The ability to decompose crude oil was tested by calculating the quantitative loss of the crude oil, achieved following the utilization of more than 93% nanoparticle compared to that of 25% fungal isolation. This indicates a high efficiency of the silver nanoparticles in breaking down crude oil with a percentage that is double that of the fungal isolation[40].

Storability of the Silver Nanoparticle

The bio-nanoparticle was stored for six months in cooling, after which the phenotypic changes of the crude oil due to the effectiveness of the silver nanoparticles were studied by adding the stored nanoparticles to the crude oil sample in water. The results were compared with the crude oil sample (the silver nanoparticles-free control) in water for one week of incubation at room temperature. The appearance of crude oil emulsification was observed as a result of the effectiveness of the nanoparticle, as well as the absence of phenotypic changes in response to the silver nanoparticles stored for six months.

Singh et al, [41] found one characteristic feature of AgNPs produced by *Cedecea* sp. extracts which is their extreme stability. Inductively coupled plasma mass spectrometry and thermogravimetric analysis revealed that the produced AgNPs are stable for periods exceeding one year.

The Effectiveness of the Silver Nanoparticle in Decomposing Oil Pollutants

The chemical analysis of the samples under study was performed utilizing GC-MASS chromatography and compared to those of the non-biological (control) sample. The results revealed the appearance of 55 graphic peaks, each peak refers to a chemical compound in the control sample (untreated crude oil). Nevertheless, a large number of these peaks (51 peaks) disappeared and only 4 peaks appeared when examining the sample of crude oil-contaminated water treated with silver nanoparticles, but the area of these peaks was greatly reduced as shown in Figs. 10,11 and 12, Tables 1 and 2. This possibly refers to a significant reduction in the concentration of compounds, which indicates the efficiency of this method in decomposing hydrocarbon compounds [42]. Previous studies indicated the use of this technique to detect the dissociation occurring in petroleum compounds and the identification of the kind and concentration of those decomposing compounds utilizing the GC-MASS technique [43].

2024, 21(6): 1935-1952
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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

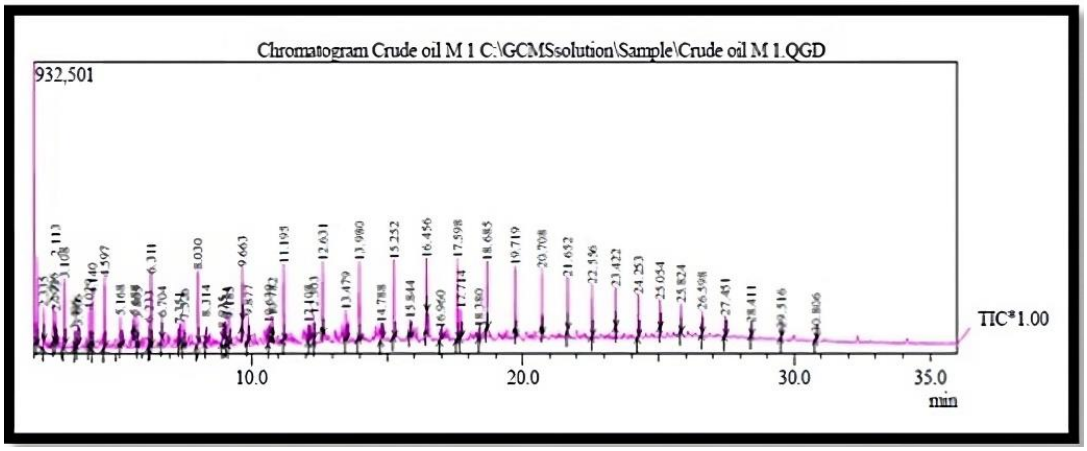


Figure 10. GC-MASS results of the control sample of untreated the crude oil showing the retention time and peaks of the chemical compounds.

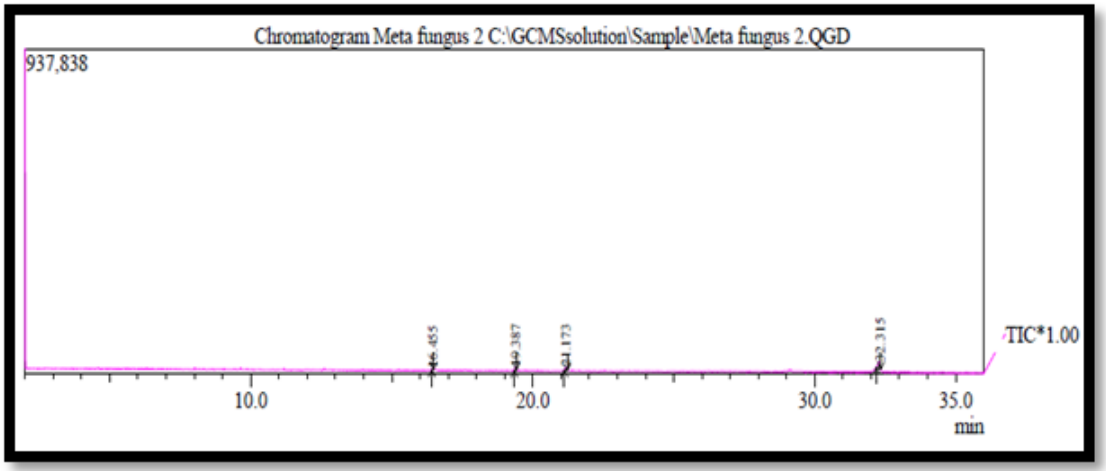


Figure 11. GC-MASS results of a sample of the crude oil treated with silver nanoparticles, showing the decrease in the number and area of the chemical compounds' peaks.

(A) (B)

Figure 12. The phenotypic changes of crude oil decomposed by the treatment with the silver nanoparticle, indicating the occurrence of a clear biodegradation.

A) treated crude oil with colloidal solution of silver nanoparticles.

B) untreated crude oil (control).

2024, 21(6): 1935-1952
https://doi.org/10.21123/bsj.2023.8577
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

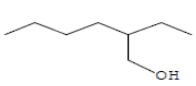
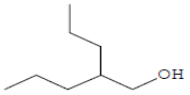
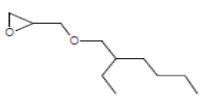
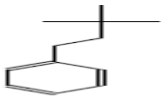
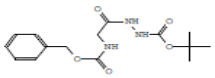
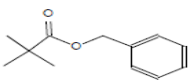
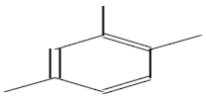
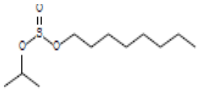
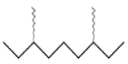
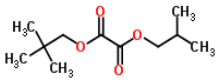
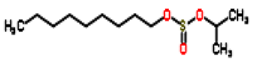
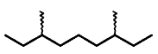
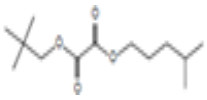
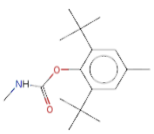
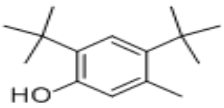
Table 1. Results of gas chromatography-mass test of crude oil samples before and after nanoparticle treatment.

crude oil samples before nanoparticle treatment.			**crude oil samples after nanoparticle treatment.**		
Peak	**Ret. Time**	**Area**	**Peak**	**Ret. Time**	Area
1	2.113	**392225**	**1**	16.455	**8166**
2	**2.335**	174845	**2**	**19.387**	20517
3	2.706	**275077**	**3**	**21.173**	24393
4	2.793	123804	**4**	32.315	**111324**
5	**3.108**	411692			164400
6	3.496	**95114**			
7	3.606	**125971**			
8	**4.029**	**244902**			
9	**4.140**	**355360**			
10	4.597	**443294**			
11	**5.168**	**192731**			
12	**5.688**	**66086**			
13	**5.805**	**149389**			
14	**6.233**	**122742**			
15	6.311	**489170**			
16	**6.704**	111700			
17	**7.351**	91567			
18	7.526	158859			
19	**8.030**	**523704**			
20	**8.314**	**64827**			
21	8.935	**103769**			
22	**9.074**	108961			
23	9.185	150919			
24	**9.663**	331473			
25	**9.877**	**131664**			
26	**10.639**	**104660**			
27	10.782	58517			
28	**11.195**	**557228**			
29	**12.108**	**85239**			
30	**12.303**	**129824**			
31	**12.631**	**448940**			
32	**13.479**	**171524**			
33	**13.980**	**585619**			
34	**14.788**	**134532**			
35	15.252	**518203**			
36	15.844	**102329**			
37	**16.456**	**339648**			
38	**16.960**	117132			
39	17.598	**515348**			
40	**17.714**	**188511**			
41	**18.380**	**52815**			
42	**18.685**	355167			
43	**19.719**	**375842**			
45	**20.708**	312464			
45	21.652	**283634**			
46	**22.556**	**296245**			
47	**23.422**	183491			
48	24.253	**252713**			
49	**25.054**	**129363**			
50	25.824	**137232**			
51	26.598	**136835**			
52	**27.451**	**131174**			
53	**28.411**	**74556**			

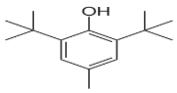
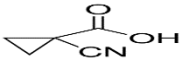
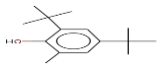
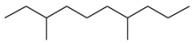
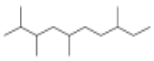
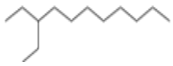
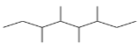
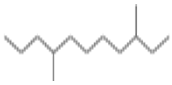
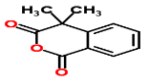
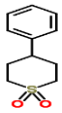
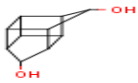
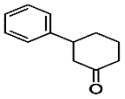
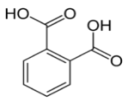
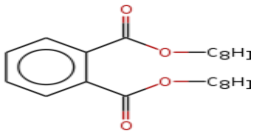
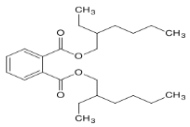
2024, 21(6): 1935-1952
https://doi.org/10.21123/bsj.2023.8577
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

54	**29.516**	58882
55	30.806	**56377**
		12333889

Table 2. Hydrocarbon compounds dissociated by the effects of the prepared silver nanoparticles, as indicated by GC-MASS chromatography [44].

Chemical Name	Chemical Composition	Chemical Name	Chemical Composition
Methylcyclohexane		1-Methyl-1-ethylcyclopentane	
Ethylhexanol		2-Propyl-1-pentanol	
Propane, 1,2-epoxy-3-[(2-ethylhexyl)oxy]		Benzene, (3,3-dimethylbutyl)	
Acethydrazide		3-ethenyl-5,5-dimethylhexyl)	
2,2-dimethyl-, phenylmethyl ester		Benzene, 1,2,4-trimethyl	
Sulfurous acid, octyl 2-propyl ester		Nonane, 3,7-dimethyl	
Oxalic acid, isohexylneopentyl ester		Sulfurous acid, nonyl 2-propyl ester	
Sulfurous acid, decyl 2-propyl ester		Sulfurous acid, octyl 2-propyl ester	
Nonane, 3, 7-dimethyl		Oxalic acid, isohexylneopentyl ester	
Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl, methylcarbamate		4,6-di-tert-butyl-m-cresol	

2024, 21(6): 1935-1952
https://doi.org/10.21123/bsj.2023.8577
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Butylated hydroxytoluene		Cyclopropanecarboxylic acid,1-hydroxy	
Phenol,4-6di(1,1dimethylethyl)2-methyl		Decane,3,7-dimethyl	
Decane,2,3,5,8-tetramethyl		Undecane ,3-ethyl	
Octane,3,4,5,6-tetramethyl		Undecane,3,8-dimethyl	
Benz [c] pyran-1,3-dione, 4,4-dimethyl		3-phenylthiane,s,s-dioxide	
1,2,4-metheno-1H-cyclobuta[cd]pentalene-3,5-diol, octahydro		Cyclohexanone, 3-phenyl	
1,2-Benzenedicarboxylic acid		1,2 Benezenedicarboxylic acid, diisooctyl ester	
Bis (2-ethylhexyl) phthalate			

Conclusion

Preparation and identification of silver nanoparticles from the extract of the fungus *Metarhizium anisopliae* was made to increase the treatment efficiency of oil and hydrocarbon pollutants in water. The utilization of an extract of a local fungal isolate that is not harmful to the environment and available locally.

Production of a nano-scale biological from the extract of *Metarhizium anisopliae* was conducted for the first time, locally in Iraq and internationally, with a decomposition efficiency of oil pollutants that is much higher than that of fungal isolation alone. The product is of high economic feasibility, material availability, cost-effectiveness, and ease of preparation.

The capability of the nanoparticles in maintaining their effectiveness under cryogenic storage conditions for six months was tested and compared to that of the fungal isolate before the production of the silver nanoparticle. The results showed high efficiency and good storage capacity.

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Baghdad Science Journal

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Ministry of Science and Technology, Directorate of Environment and Water.

Authors' Contribution Statement

Each of the authors contributed to the writing of the manuscript, and the work was distributed as follows: M. T. A. designed the presented idea and prepared silver nanoparticles, Z. Z. A. handled the data and tracked the process of auditing the search. M. T. A., S. I. M., and U. F. R. made the sample analysis.

References

1. Alexander M, Engel LS, Olaiya N, Wang L, Barrett J, Weems L, et al. The deepwater horizon oil spill coast guard cohort study: A cross-sectional study of acute respiratory health symptoms. Environ Res. 2018; 162: 196-202. https://doi.org/10.1016/j.envres.2017.11.044
2. Amir-Heidari P, Arneborg L, Lindgren JF, Andreas L, Rosen L, Raie M, et al. state-of-the-art model for spatial and stochastic € oil spill risk assessment: a case study of oil spill from a shipwreck. Environ Int. 2019; 126: 309e320. https://doi.org/10.1016/j.envint.2019.02.037
3. Mohammed DB, Abbas AH, Ali AMA, Abed EH. Biodegradation of Anthracene Compound by Two Species of Filamentous Fungi. Baghdad Sci J. 2018; 5(1): 43-47. http://dx.doi.org/10.21123/bsj.2018.15.1.0043
4. Hauptfeld E, Pelkmans J, Huisman TT, Anocic A, Snoek BL, von Meijenfeldt FAB, et al. A metagenomic portrait of the microbial community responsible for two decades of bioremediation of poly-contaminated groundwater. Water Res. 2022; 221:118767. https://doi.org/10.1016/j.watres.2022.118767
5. Dhar K, Subashchandrabose SR, Venkateswarlu K, Krishnan K, Megharaj M. Anaerobic Microbial Degradation of Polycyclic Aromatic Hydrocarbons: A Comprehensive Review, in: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology . Springer International Publishing, Cham. 2020; 251 pp. 25–108. https://doi.org/10.1007/398_2019_29.
6. Liu Q, Tang J, Gao K, Gurav R, Giesy JP. Aerobic degradation of crude oil by microorganisms in soils from four geographic regions of China. Sci Rep. 2017; 7: 14856. https://doi.org/10.1038/s41598-017-14032-5.
7. Hasson SO, Salman SAk, Hassan SF, Abbas SM. Antimicrobial Effect of Eco- Friendly Silver Nanoparticles Synthesis by Iraqi Date Palm (Phoenix dactylifera) on Gram-Negative Biofilm-Forming Bacteria. Baghdad Sci J. 2021; 18(4): 1149-1156. http://dx.doi.org/10.21123/bsj.2021.18.4.1149.
8. Alsharari SS, Alenezi MA, Al Tami MS, Soliman M. Recent advances in the Biosynthesis of Zirconium Oxide Nanoparticles and their Biological Applications. Baghdad Sci J. 2023; 20(1): 41-57. https://dx.doi.org/10.21123/bsj.2022.7055
9. Shah M, Nawaz S, Jan H, Uddin N, Ali A, Anjum S, et al. Synthesis of bio-mediated silver nanoparticles from Silybum marianum and their biological and clinical activities. Mater Sci Eng C Mater Biol Appl. 2020; 112: 110889. https://doi.org/10.1016/j.msec.2020.110889.
10. Abd-Elhady HM, Ashor MA, Hazem A, Saleh FM, Selim S, El Nahhas N, et al. Biosynthesis and Characterization of Extracellular Silver Nanoparticles from Streptomyces aizuneusis: Antimicrobial, Anti Larval, and Anticancer Activities. Molecules. 2021; 27(1): 212. https://doi.org/10.3390/molecules27010212.
11. Khalafi T. Phycosynthesis and Enhanced Photocatalytic Activity Of Zinc Oxide Nanoparticles Toward Organosulfur Pollutants. Scie Rep. 2019; 9: 6866. https://doi.org/10.1038/s41598-019-43368-3
12. Ummidi V, Ravi S, Padmaja V. Preparation and use of oil formulations of Beauveria bassiana and Metarhizium anisopliae against Spodoptera litura larvae. Afr J Microbi Res. 2014; 8(15): 1638-1644. https://doi.org/10.5897/AJMR2013.6593
13. Rodriguez-Tudela JL, Arendrup MC, Barchiesi F, Bille J, Chryssanthou E, Cuenca-Estrella M, et al. EUCAST Definitive Document EDef 7.1: method for

the determination of broth dilution MICs of antifungal agents for fermentative yeasts: Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). Clin Microbiol Infect. 2008; 14(4): 398-405. https://doi.org/10.1111/j.1469-0691.2007.01935.x

14. Wijdan AA, Bahrouz MAA, Sajid SSA. Cultivation and Detection of Unculturable Fungi in Soil Using Soil Infusion Agar (SIA). J univ Al-anbar pure sci . 2018; 12(1): 9-18 https://www.iasj.net/iasj/article/152280
15. Silva WOB, Mitidieri S, Schrank A, Vainstein MH. Production and extraction of an extracellular lipase from the entomopathogenic fungus Metarhizium anisopliae. Process Biochem. 2005; 40(1): 321-326. https://doi.org/10.1016/j.procbio.2004.01.005
16. Labeeb AA, Marwah TA, Zahraa ZA, Manal MA. Biodegradation of chlorpyrifos pesticide using silver bio-nanoparticles Bacillus thuringiensis israelensis extracts. IOP Conf. Ser.: Earth Environ Sci. 2021; 779: 012113 https://doi.org/10.31830/2348-7542.2019.138
17. Rheder DT, Guilger M, Bilesky-José N, Germano-Costa T, Pasquoto-Stigliani T, Gallep TBB, et al. Synthesis of biogenic silver nanoparticles using Althaea officinalis as reducing agent: evaluation of toxicity and ecotoxicity. Sci Rep. 2018; 8(1): 12397. https://doi.org/10.1038/s41598-018-30317-9
18. Osorio-Echavarría J, Osorio-Echavarría J, Ossa-Orozco CP, Gómez-Vanegas NA. Synthesis of silver nanoparticles using white-rot fungus Anamorphous Bjerkandera sp. R1: Influence of silver nitrate concentration and fungus growth time. Sci Rep. 2021; 11(1): 3842. https://doi.org/10.1038/s41598-021-82514-8
19. Skanda S, Bharadwaj PSJ, Datta Darshan VM, Sivaramakrishnan V, Vijayakumar BS. Proficient mycogenic synthesis of silver nanoparticles by soil derived fungus Aspergillus melleus SSS-10 with cytotoxic and antibacterial potency. J Microbiol Methods. 2022; 199: 106517. https://doi.org/10.1016/j.mimet.2022.106517
20. Tyagi PK, Mishra R, Khan F, Gupta D, Gola D. Antifungal effects of silver nanoparticles against various plant pathogenic fungi and its safety evaluation on *Drosophila melanogaster*. Biointerface Res Appl Chem. 2020; 10: 6587-6596. https://doi.org/10.33263/BRIAC106.65876596
21. Oudot J. Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry. Marine Environ Res. 1984; 13(4): 277-302. https://doi.org/10.1016/0141-1136(84)90034-5
22. Al-Zaban MI, Mahmoud MA, AlHarbi MA, Bahatheq AM. Bioremediation of Crude Oil by Rhizosphere Fungal Isolates in the Presence of Silver Nanoparticles. Int J Environ Res Public Health. 2020; 17(18): 6564. https://doi.org/10.3390/ijerph17186564.
23. Alnafisah AS, Alqrairy E, Tar H, M Alminderej F, Aroua LM, Graff B, et al. Light-Assisted Synthesis of Silver and Gold Nanoparticles by New Benzophenone Derivatives. ACS Omega. 2023 Jan 11; 8(3): 3207-3220. https://doi.org/10.1021/acsomega.2c06655.
24. Talabani RF, Hamad SM, Barzinjy AA, Demir U. Biosynthesis of Silver Nanoparticles and Their Applications in Harvesting Sunlight for Solar Thermal Generation. Nanomaterials (Basel). 2021 Sep 17; 11(9): 2421. https://doi.org/10.3390/nano11092421.
25. Inès Hammami a, Nadiyah M. Alabdallah a, Amjad Al jomaa a, Madiha kamoun. Gold nanoparticles: Synthesis properties and applications. J King Saud Univ Sci. 2021; 33(7): 101560. https://doi.org/10.1016/j.jksus.2021.101560
26. Sonbol H, Mohammed AE, Korany SM. Soil Fungi as Biomediator in Silver Nanoparticles Formation and Antimicrobial Efficacy. Int J Nanomed. 2022; 17: 2843-2863. https://doi.org/10.2147/IJN.S356724
27. Ammar HA, El-Desouky TA. Green synthesis of nanosilver particles by Aspergillus terreus HA1N and Penicillium expansum HA2N and its antifungal activity against mycotoxigenic fungi. J Appl Microbiol. 2016 Jul; 121(1): 89-100. https://doi.org/10.1111/jam.13140.
28. Durán N, Marcato PD, Alves OL, De Souza GI, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Nanobiotechnology 2005 Jul;3: 1-7.
29. Honary S, Barabadi H, Gharaei-Fathabad E, Naghibi F. Green synthesis of silver nanoparticles induced by the fungus Penicillium citrinum. Tropical J Pharma Res. 2013; 12(1): 7-11. https://doi.org/10.4314/tjpr.v12i1.2
30. Bocate KP, Reis GF, de Souza PC, Oliveira Junior AG, Durán N, Nakazato G, et al. Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of *Aspergillus*. Int J Food Microbiol. 2019; 291:79-86. https://doi.org/10.1016/j.ijfoodmicro.2018.11.012.
31. Phanjom P, Ahmed G. Biosynthesis of silver nanoparticles by Aspergillus oryzae (MTCC No. 1846) and its characterizations. J. Nanosci and Nanotechnol. 2015; 5 (1): 14-21. https://doi.org/10.5923/j.nn.20150501.03.
32. Lengke MF, Fleet ME, Southam G. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver(I) nitrate complex. Langmuir. 2007; 23(5): 2694-9. https://doi.org/10.1021/la0613124.

33. Priyadarshini E, Priyadarshini SS, Cousins BG, Pradhan N. Metal-Fungus interaction: Review on cellular processes underlying heavy metal detoxification and synthesis of metal nanoparticles. Chemosphere. 2021; 274: 129976. https://doi.org/10.1016/j.chemosphere.2021.129976
34. Dada AO, Adekola FA, Dada FE, Adelani-Akande AT, Bello MO, Okonkwo CR, et al. Silver nanoparticle synthesis by Acalypha wilkesiana extract: phytochemical screening, characterization, influence of operational parameters, and preliminary antibacterial testing. Heliyon. 2019; 5(10): e02517. https://doi.org/10.1016/j.heliyon.2019.e02517
35. Elamawi RM, Al-Harbi RE, Hendi AA. Biosynthesis and characterization of silver nanoparticles using Trichoderma longibrachiatum and their effect on phytopathogenic fungi. Egypt J Biol Pest Control. 2018; 28(1): 1-11. https://doi.org/10.1186/s41938-018-0028-1
36. AbdelRahim K, Mahmoud SY, Ali AM, Almaary KS, Mustafa AE, Husseiny SM. Extracellular biosynthesis of silver nanoparticles using Rhizopus stolonifer. Saudi J Biol Sci. 2017; 24(1): 208-216. https://doi.org/10.1016/j.sjbs.2016.02.025
37. Soleimani P, Mehrvar A, Michaud JP, Vaez N. Optimization of silver nanoparticle biosynthesis by entomopathogenic fungi and assays of their antimicrobial and antifungal properties. J Invertebr Pathol. 2022; 190: 107749. https://doi.org/10.1016/j.jip.2022.107749
38. Tyagi S, Tyagi PK, Gola D, Chauhan N, Bharti RK. Extracellular synthesis of silver nanoparticles using entomopathogenic fungus: Characterization and antibacterial potential. SN Appl Sci. 2019; 1: 1545. https://doi.org/10.1007/s42452-019-1593-y.
39. Abdellatif AAH, Alturki HNH, Tawfeek HM. Different cellulosic polymers for synthesizing silver nanoparticles with antioxidant and antibacterial activities. Sci Rep. 2021; 11(1): 84. https://doi.org/10.1038/s41598-020-79834-6
40. Mustafa AA, Shaymaa AA, Hamid TA. Biodegradation of crude oil using Aspergillus species. J. Biol Agric Healthcare. 2019; 9(4): 60-64. https://doi.org/10.7176/JBAH
41. Singh P, Pandit S, Jers C, Joshi AS, Garnæs J, Mijakovic I. Silver nanoparticles produced from Cedecea sp. exhibit antibiofilm activity and remarkable stability. Sci Rep. 2021; 11(1): 12619. https://doi.org/10.1038/s41598-021-92006-4
42. Novianty R, Hidayah A, Saryono S, Awaluddin A, Pratiwi Nw, Juliantari E. The diversity of fungi consortium isolated from polluted soil for degrading petroleum hydrocarbon. Biodiversitas. 2021; 22 (11): 5077-5084. https://doi.org/10.13057/biodiv/d221145.
43. Bakri M. Assessing some Cladosporium species in the biodegradation of petroleum hydrocarbon for treating oil contamination. J Appl Microbiol. 2022; 133(6): 3296-3306. https://doi.org/10.1111/jam.15815
44. Merv Fingas. Introduction to Oil Chemistry and Properties Related to Oil Spills. H. Wilkes (ed.), Hydrocarbons, Oils and Lipids: Diversity, Origin, Chemistry and Fate, Handbook of Hydrocarbon and Lipid Microbiology, Springer Nature Switzerland AG 2020; 1-32. https://doi.org/10.1007/978-3-319-54529-5_29-1

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إنتاج جسيمات الفضة النانوية المُصنَّعة حيوياً من فطر *Metarhizium anisopliae* لمعالجة الملوثات النفطية في الماء

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الخلاصة

في هذه الدراسة تم استخدام طريقة حيوية لتحضير جزيئات الفضة النانوية (AgNPs) من مستخلص فطر *Metarhizium anisopliae*. تم التعرف على خصائص AgNPs المحضرة باستخدام جهاز تحليل امتصاصية الطيف بالأشعة الضوئية والاشعة فوق البنفسجية، جهاز التحليل الطيفي بالأشعة تحت الحمراء. تم تحديد شكل وحجم وتوزيع الشحنات على جسيمات الفضة النانوية باستخدام المجهر الالكتروني الماسح وتحليل جهد زيتا. أظهر تحليل النشاط الحيوية لجسيمات الفضة النانوية فعاليتها في معالجة الملوثات بنسبة أكثر من 93 % من وزن نفط الخام في عينات المياه الملوثة. اذ تحولت كتلة النفط الخام في الماء بشكل فعال إلى كتلة هلامية فاقدة لقوامها واستحلابها، مقارنة بعينة النفط الخام (السيطرة)، بعد سبعة أيام من الحضانة عند درجة حرارة 28 ± 2 م°. تم إجراء التحليل الكيميائي لعينات المياه الملوثة بالنفط الخام المعالجة وغير المعالجة بالدقائق النانوية باستخدام تقنية كروماتوغرافيا الغاز- الكتلة (GC-MASS chromatography). أظهرت النتائج ظهور 55 قمة بيانية (كل قمة بيانية تشير الى مركب كيميائي) في نموذج السيطرة بينما اختفت 51 قمة من هذه القمم واختزلت مساحة 4 قمم الأخرى. تم اختبار قدرة جسيمات الفضية النانوية في الحفاظ على فعاليتها في ظل ظروف التخزين المبردة لمدة ستة أشهر ومقارنتها بقدرة العزلة الفطرية قبل إنتاج جسيمات الفضة النانوية. أظهرت النتائج عدم وجود تغيرات معنوية في حجم وشكل وكفاءة جزيئات الفضة النانوية في معالجة الملوثات النفطية في الماء. اشارت النتائج إلى الكفاءة العالية لجسيمات الفضة النانوية مقارنة بالمواد الكيميائية في معالجة الملوثات البترولية وكذلك تعزيز قابلية الذوبان والاستحلاب وتفكك الهيدروكربونات. فضلاً عن، تتميز جزيئات الفضة النانوية بكلف انتاج قليلة وتوفر مواد الانتاج رخيصة الثمن وسهولة وسرعة انتاجها وصديقة للبيئة مقارنة باستخدام المنتجات الكيميائية ذات سمية العالية للأحياء المائية وغالية الثمن وكلفة انتاجيتها العالية فضلاً عن تراكمها في النظام البيئي (غير امنة بيئيا).

الكلمات المفتاحية: جسيمات الفضة النانوية، التحلل الحيوي، التكنولوجيا الحيوية، فطر *Metarhizium anisopliae*، الملوثات البترولية.