



Research Article

Study of the biosynthesis of gold nanoparticles by using several saprophytic fungi



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Received: 15 July 2020 / Accepted: 14 October 2020 / Published online: 21 October 2020
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Abstract

Gold nanoparticles have wide applications, such as cancer diagnosis and treatment, drug transfer, gene transfer, and DNA and protein detection. Fungi are good options for the biosynthesis of nanoparticles, which is due to their ability to secrete a large quantity of enzymes. The purpose of the present study is to investigate the ability of several saprophytic fungi in the production of gold nanoparticles. The biomass of each fungus was first mixed with a 10^{-3} molar HAuCl_4 solution and was subsequently heated for 24 h. The Change in the color of the reaction solutions from yellow to violet was symptomatic of the synthesis of gold nanoparticles. In cases where the above assessment (i.e., color change) was positive, the synthesis of gold nanoparticles was further investigated by using spectrophotometry UV–vis, X-ray diffraction and TEM electron microscope. The observation of the maximum absorption peak at a wavelength of about 540 nm in the UV–vis spectrum confirmed the production of gold nanoparticles. Moreover, the XRD analysis of the nanoparticles proved that the nanoparticles synthesized are gold nanocrystals. The TEM electron microscope technique was used to determine the shape and size distribution of the nanoparticles. Based on electromicrographs, the nanoparticles were spherical, triangular and polygonal.

Keywords Fungi · Gold nanoparticles · Synthesis · TEM · XRD

1 Introduction

Nanotechnology is one of the growing branches of science and involves the production, manipulation and the use of materials at a scale smaller than microns. The properties of materials in nano and micro scales are not identical. The synthesis of nanomaterials is important due to their unusual optical, chemical and electrical properties. Gold nanoparticles are widely used in medical fields such as cancer diagnosis and treatment, drug treatment, biosensors, DNA and protein detection, and the removal of pathogenic microorganisms from contaminated water. Although nanoscale materials are mainly produced by chemical industries, living organisms are an important

source of these materials. This is because chemical methods are time-consuming and costly. Nanoparticles can be produced by bacteria, actinomycetes, fungi, and plants. Throughout the world, numerous studies have been conducted which employ microorganisms such as bacteria and actinomycetes (prokaryotes), as well as algae, yeasts, fungi, and organic plants (eukaryotes) for the synthesis of metal nanoparticles. Due to Fungi's simple and inexpensive cultivation in both industrial and laboratory scales, their release of enzymes, safety for the environment, easy manipulation, the ability to resuscitate one or more metal ions, the production of high volumes of nanoparticles and relatively lower cost, they are usually preferred to other microorganisms.

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In recent studies, fungi such as *Fusarium*, *Verticillium*, and *Aspergillus* have been used to synthesize metal nanoparticles such as copper, cadmium, silver, and gold [1]. In the vicinity of gold ions, fungi produce large quantities of nanoparticles. Mukherjee et al. [2] employed the fungus *Verticillium* sp. to synthesize gold nanoparticles. The maximum absorption peak in the UV–vis diagram was 540 nm. In addition, they reported average size of 20 nm for nanoparticles. They also used the fungus *Fusarium oxysporum* for extracellular synthesis of gold nanoparticles [3]. The UV–vis spectrometry showed the maximum absorption peak at 545 nm. Additionally, they reported that the synthesized nanoparticles were spherical or triangular and fall within the range of 8–40 nm. By using the endophytic fungus *Colletotrichum* which was isolated from the geranium leaves, Shankar et al. [4] produced gold nanoparticles. Ahmad et al. [5] also employed the fungus *Trichothecium* to produce gold nanoparticles. They reported that by controlling the reaction conditions, the synthesis of gold nanoparticles can be intracellular or extracellular. Bhambure et al. [6] employed the fungus *Aspergillus niger* for extracellular biosynthesis of gold nanoparticles. The average size of the produced nanoparticles was 12.79 ± 5.61 nm. They found that the synthesized nanoparticles are very stable in the solution. Philip [7] produced Au, Ag and Au–Ag nanoparticles by employing the extract of *Volvariella volvacea*. The shape of the nanoparticles was triangular, spherical and hexagonal. Moreover, their dimensions were in the range of 20–150 nm and the morphology of the nanoparticles was dependent on the extract temperature. At the lower temperature, the nanoparticles were almost hexagonal while at the higher temperature dendrite/fractal structures were observed. Binupriya et al. [8] used the fungus *Rhizopus stolonifer* for biosynthesis of gold and silver nanoparticles. The formation of nanoparticles was confirmed by UV–vis spectrum, TEM and XRD analyses. The gold nanoparticles were in the range of 25–30 nm. Soni and Prakash [9] used the fungus *Aspergillus niger* to synthesize gold nanoparticles. The UV–vis spectrometry showed the maximum absorption peak at 530 nm. In addition, the size of the nanoparticles was in the range of 10–30 nm. Sarkar et al. [10] produced gold nanoparticles by using the fungus *Alternaria alternata*. By employing the Fourier transform infrared spectroscopy, the existence of a protein shell outside the nanoparticles was confirmed which had a supporting role for their stability. By using the fungus *Fusarium oxysporum*, Thakker et al. [11] performed a fast extracellular synthesis of gold nanoparticles. The average size of the nanoparticles was 22 nm, and these nanoparticles exhibited antimicrobial activity against *Pseudomonas* sp.. Gopinath and Arumugam [12] reported that the fungus *Fusarium solani* can be used for the fast extracellular synthesis of gold nanoparticles. The size of the nanoparticles ranged from 20–50 nm. Joshi et al. [13] employed the marine endophytic fungus *Cladosporium*

cladosporioides for the synthesis of gold nanoparticles with a mean size of 100 nm and reported strong antioxidant and antibacterial properties for them. Patil and Kim [14], utilizing marine microorganisms such as bacteria, fungi, and microalgae, produced nanoparticles. For instance, the fungi *Aspergillus sydowii* and *Aspergillus terreus* had the capability of producing gold nanoparticles. Pourali et al. [15] considered the biosynthesis of gold nanoparticles by *Fusarium oxysporum* and focused on the possibility of nanoparticles conjugation with β -Lactam antibiotics. They reported high tendency of conjugation with antibiotics for nanoparticles. Sreedharan et al. [16] employed the fungus *Macrophomina phaseolina* for synthesis of gold nanoparticles. The formation of nanoparticles was confirmed by UV–vis spectrum, TEM and EDX analyses. The sizes of the nanoparticles were in the range 14–16 nm.

Based on the literature review, many studies have been carried out into the synthesis of gold nanoparticles by employing fungi. However, the ability of some fungi in the synthesis of gold nanoparticles still remains unknown. In this study, the ability of 17 fungi in the synthesis of gold nanoparticles is evaluated. In the first stage, the production of gold nanoparticles is distinguished by the color change of the mixture of biomass and chlorine solution. Subsequently, out of the fungi which are able to produce nanoparticles, 5 fungi are selected for further analysis. The analyses include UV–vis spectrometry, transient electron microscope (TEM) and X-ray diffraction. In the present study, in one case (*Verticillium dahliae*), the species of the selected fungus is different from those in the previous studies. In addition, to the best knowledge of the authors, the fungus *Rhizoctonia solani* has not been employed for the synthesis of gold nanoparticles in the published literature.

2 Materials and Methods

Tables 1 and 2 represent the material and the fungi used in this study, respectively. The fungi were first cultured on a Potato Dextrose Agar (PDA) medium. Subsequently, they were incubated at 27° C for 3 to 7 days. These cultures were used to produce fungal biomasses in a Glucose Yeast Peptone (GYP) liquid culture medium using a rotary shaker with rpm of 120 for a period of 5–7 days. After this period which resulted in well fungal growth, the biomasses were collected and well rinsed with distilled water by using Whatman filter paper No. 1 and a glass funnel to exclude the remains of

Table 1 The list of the used materials

No.	Material
1	HAuCl ₄ solution
2	PDA culture medium
3	GYP culture medium

Table 2 The list of the studied fungi

No	Fungus
1	<i>Fusarium oxysporum</i>
2	<i>Sclerotinia sclerotium</i>
3	<i>Aspergillus flavus</i>
4	<i>Paecilomyces variotii</i>
5	<i>Colletotrichum gloeosporioides</i>
6	<i>Rhizoctonia solani</i>
7	<i>Verticillium dahliae</i>
8	<i>Botrytis oryzae</i>
9	<i>Stemphillum</i> sp.
10	<i>Peceaiomyces</i> sp.
11	<i>Gaeumanomyces</i> sp.
12	<i>Metarhizium</i> sp.

the culture medium. To ensure the exclusion of secondary metabolites and culture media, the samples then subjected to three cycles of centrifugation. Each cycle runs for 10 min at 10,000 rpm. At the end of each cycle, the supernatant discarded and the pellet suspended in distilled water. Suspension of biomasses in water was used for the evaluation of their bioactivity in the production of gold nanoparticles. To this end, four samples were prepared as follows. To the first tube (control), for 0.5 g of the wet biomass, 2 ml distilled water was added. To the second tube, for half a gram of the dead fungal biomass (the fungus biomass is killed by the autoclave), 2 ml HAuCl_4 solution with a concentration of 10^{-3} molar was added. To the third tube, for half a gram of the wet biomass, 2 ml HAuCl_4 solution with a concentration of 10^{-3} molar was added. Finally, to the fourth tube for 1 ml of the culture medium (after passing from the filter paper), 2 ml of HAuCl_4 solution with a concentration of 10^{-3} molar was added. These tubes were placed inside a rotary shaker with a revolution of 120 rpm for 24–72 h at a temperature of 27° C.

3 Results and Discussion

3.1 Initial evaluation of the synthesis of gold nanoparticles

The simplest method for the purpose of the evaluation of the synthesis of gold nanoparticles is the observational method. The synthesis of gold nanoparticles is recognized by the color change of the mixture of biomass and HAuCl_4 from yellow to violet. This color change is due to the vibrational excitation of the surface plasmon by the nanoparticles, which is within the visible wavelength range and near 540 nm [17]. In the current study, 12 fungi (as listed in Table 2) were evaluated. The color change was observed

for 8 fungi. Based on this evaluation, it was concluded that only the fungi *Stemphillum*, *Metarhizium*, *Gaeumanomyces*, and *Botrytis oryzae* are not capable of synthesizing gold nanoparticles. The evaluation results for four selected fungi have been presented in Fig. 1. In each frame, from left to right, the tubes contain the live fungus biomass in the distilled water (control tube), the dead fungus biomass incubated by HAuCl_4 , the live fungus biomass incubated by HAuCl_4 and the PDA medium incubated by HAuCl_4 . In comparison with the control tube, the change in the color of colonies after 24 h at a temperature of 29 °C can be observed in Fig. 1.

3.2 UV–Vis Spectrometry

The UV–vis spectrometry is used to confirm the production of gold nanoparticles. In this analysis, the absorption of the ultraviolet ray at different wavelengths is considered. Since gold has an absorption spectrum in the range of 500–600 nm, the UV–vis spectrometry is considered for wavelengths in the range of 400–700 nm. For this purpose, about 1 ml of the supernatant of the reaction solution was isolated. Afterwards, its absorption spectrum was measured for the wavelengths in the range of 400–700 nm. The important evidence suggesting the formation of gold nanoparticles is the maximum absorption peak at a wavelength of about 540 nm [2]. Figures 2 and 3 show the results of the UV–vis spectrometry for the 8 fungi which passed the previous evaluation successfully. All of the graphs have a maximum absorption peak near the 540 nm wavelength.

Table 3 shows the wavelength of the maximum absorption for the examined fungi. The maximum and minimum amounts of absorption belonged to the fungus *Rhizoctonia solani* and *Peceaiomyces* sp., respectively.

3.3 Transient Electron Microscope (TEM) Analysis

Out of the 8 fungi which were able to produce gold nanoparticles, 4 fungi were selected for further analysis by the transient electron microscope (TEM). By using the TEM micrographs, it can be seen that the synthesis of the nanoparticles is intracellular or extracellular. Moreover, the shape and the size distribution of the nanoparticles are determined. Figure 4 presents the TEM micrographs for gold nanoparticles synthesized by the fungi *Fusarium oxysporum*, *Aspergillus flavus*, *Rhizoctonia solani*, and *Verticillium dahliae*. According to the TEM micrographs, the synthesized nanoparticles are intercellular.

The nanoparticles with different sizes and shapes can be observed in these figures. Based on these micrographs, the nanoparticles produced by fungi *Aspergillus flavus*, *Rhizoctonia solani* are mainly spherical whereas the

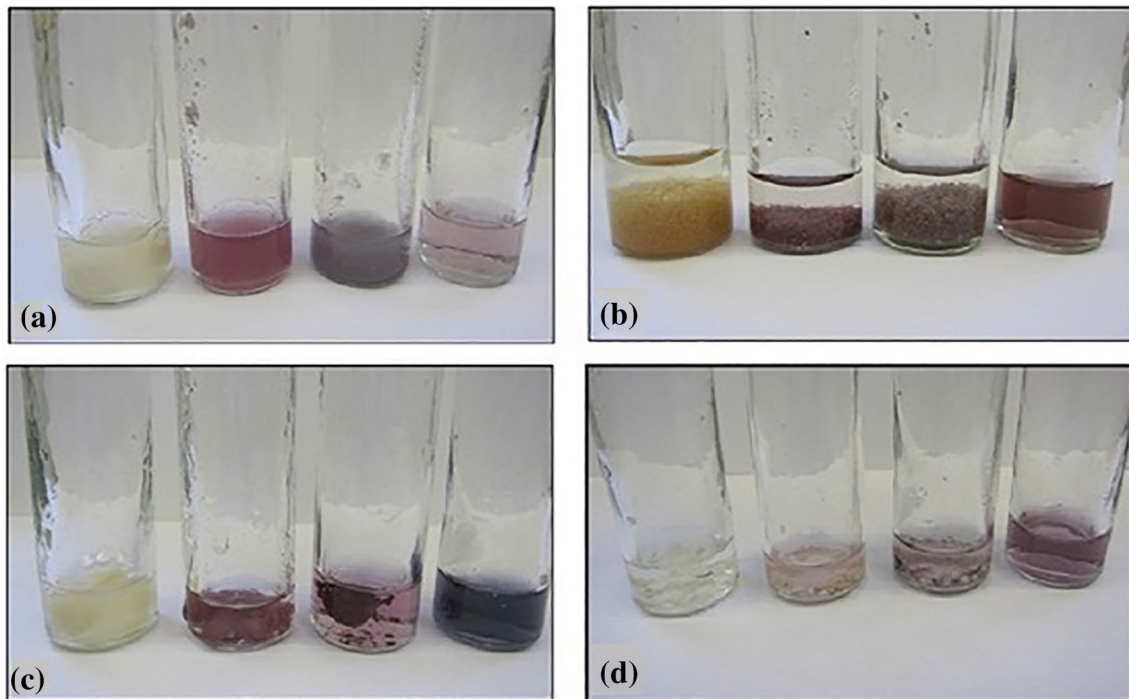


Fig. 1 The production of gold nanoparticles by the fungi **a** *Fusarium oxysporum*, **b** *Aspergillus flavus* **c** *Rhizoctonia solani*, **d** *Verticillium dahliae*

nanoparticles produced by *Fusarium oxysporum* are triangular and spherical and those produced by *Verticillium dahliae* are often polygonal. The TEM micrographs are used to determine the size distribution of gold nanoparticles. The results appear in Fig. 5 for the examined fungi. It can be observed that the maximum number of the synthesized nanoparticles belongs to the fungus *Rhizoctonia solani*. The mean size of nanoparticles for the fungi *Aspergillus flavus*, and *Rhizoctonia solani* is about 30 nm and for the fungi *Fusarium oxysporum* and *Verticillium dahliae*, it is 20 and 40 nm, respectively. Therefore, the smallest and largest sizes of the nanoparticles belong to the fungi *Fusarium oxysporum* and *Verticillium dahliae*, respectively. As it is observed, different fungi produce nanoparticles with different sizes and shapes. These differences are probably related to the kind and amount of secreted enzymes by different fungi. However, more researches are required to understand the mechanism of this phenomenon.

3.4 X-ray diffraction (XRD) Analysis

X-ray diffraction (XRD) is a direct method for the determination of the phase-type and crystalline structure of materials. To prepare for XRD analysis, the samples (after gold nanoparticle synthesis) are centrifuged with a revolution of 18,000 rpm for 20 min. Subsequently, the supernatant is discarded and the deposited nanoparticles are sent for the

XRD analysis. Figures 6 and 7 show the XRD analysis results for the examined fungi. The diagrams represent the intensity of the diffracted beam versus the diffraction angle. In all the diagrams, the peaks are observed at the diffraction angles 38.09°, 44.3°, 64.45° and 77.37°, which correspond to the crystalline plates (111), (200), (220), and (311), respectively. This analysis proves that the synthesized particles are gold nanocrystals [9]. In all the samples, before the diffraction angle 38.09°, some peaks are observed. The reason is that in addition to gold nanoparticles, the samples contain organic materials.

3.5 Verification of the Results

In this section, for the purpose of verification, the results of the present work are related to the relevant findings in the literature. Mukherjee et al. [3] synthesized gold nanoparticles by the fungus *Fusarium oxysporum*. They used the HAuCl_4 solution with a concentration of 10^{-3} M. The UV–vis spectrometry showed the maximum absorption peak at 545 nm. Moreover, they reported that the synthesized nanoparticles were spherical or triangular and in the range of 8–40 nm. In the present study, the maximum absorption peak was 530 nm. In addition, the nanoparticles were synthesized in the range of 5–41 nm, with spherical or triangular shapes. These agreements confirm the validity of our results.

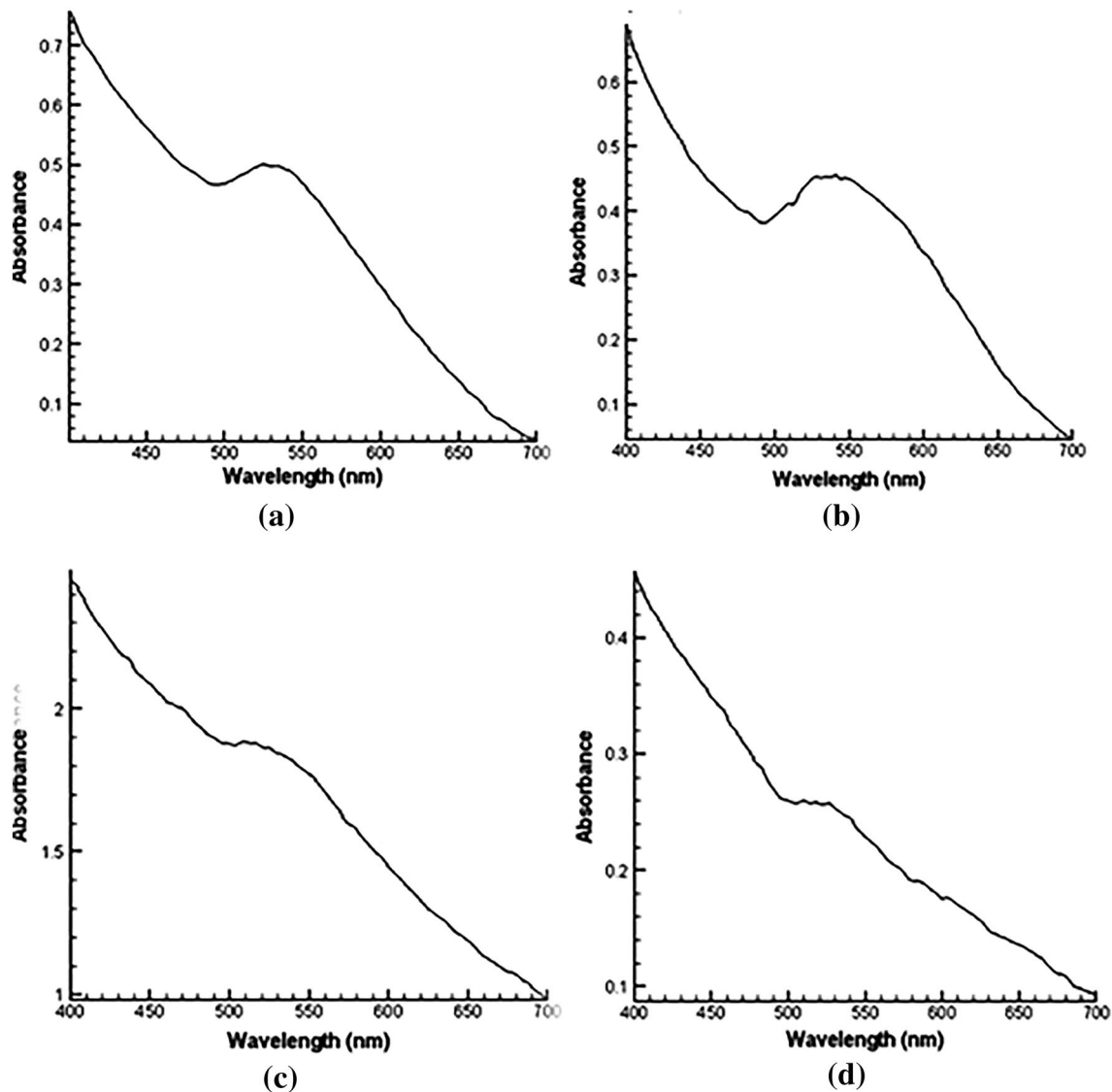


Fig. 2 The UV–vis spectrometry analysis for the fungi **a** *Fusarium oxysporum*, **b** *Aspergillus flavus*, **c** *Rhizoctonia solani*, **d** *Colletotrichum gloeosporioides*

Soni and Prakash [9] used the fungus *Aspergillus niger* to synthesize gold nanoparticles. The concentration of the HAuCl_4 solution was 10^{-3} M. The UV–vis spectrometry showed the maximum absorption peak at 530 nm. Besides, the size of the nanoparticles was in the range of 10–30 nm. In the present study, the maximum absorption peak for *Aspergillus niger* was 537. The fungus *Aspergillus flavus* was also examined in the current study. The maximum absorption peak was observed at 534 nm, and the nanoparticles were in the range of 5–51 nm. It could be seen that *Aspergillus flavus* produces larger nanoparticles, as compared with *Aspergillus niger*. Therefore, it is concluded that the species of the fungus can affect the size of the synthesized nanoparticles.

Mukherjee et al. [2] employed the fungus *Verticillium* sp. to synthesize gold nanoparticles. In their experiment, the concentration of the HAuCl_4 solution was 10^{-4} M. The maximum absorption peak in the UV–vis diagram was 540 nm. They also reported average size of 20 nm for the nanoparticles. In the study undertaken here, the experiments were carried out on the fungus *Verticillium dahliae*. The maximum absorption peak and the average size of the nanoparticles were 534 and 40 nm, respectively. In the present study, the average size of the nanoparticles is larger. This is probably due to the difference in the concentration of the HAuCl_4 solution used in the two studies. The use of a solution with higher molarity results in larger nanoparticles. Besides, Mukherjee et al. did not specify the species

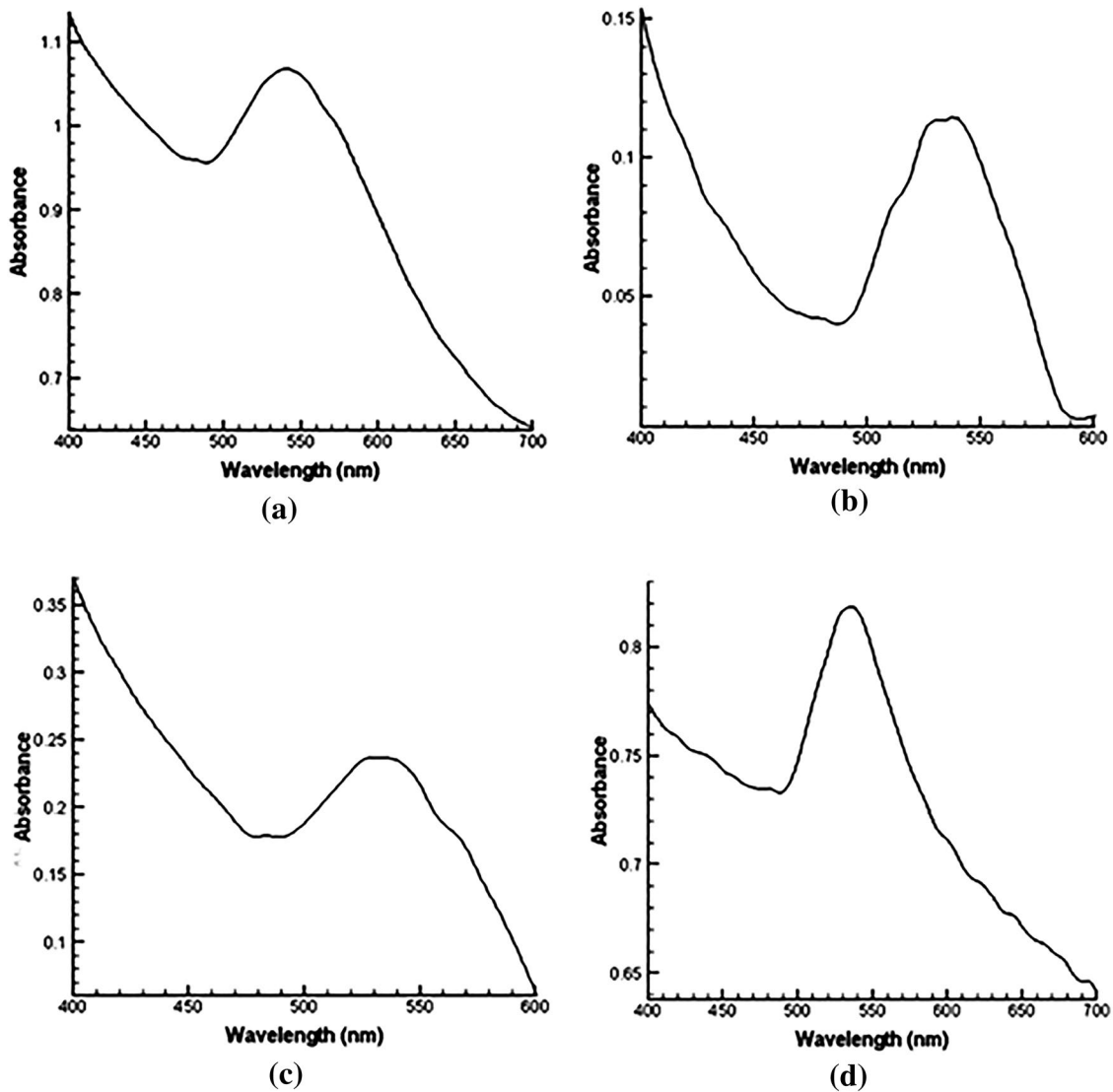


Fig. 3 The UV–vis spectrometry analysis for the fungi **a** *Paecilomyces variotii*, **b** *Peceaiomyces* sp. **c** *Sclerotinia sclerotium*, **d** *Verticillium dahliae*

Table 3 The wavelength of the maximum absorption peak for the examined fungi

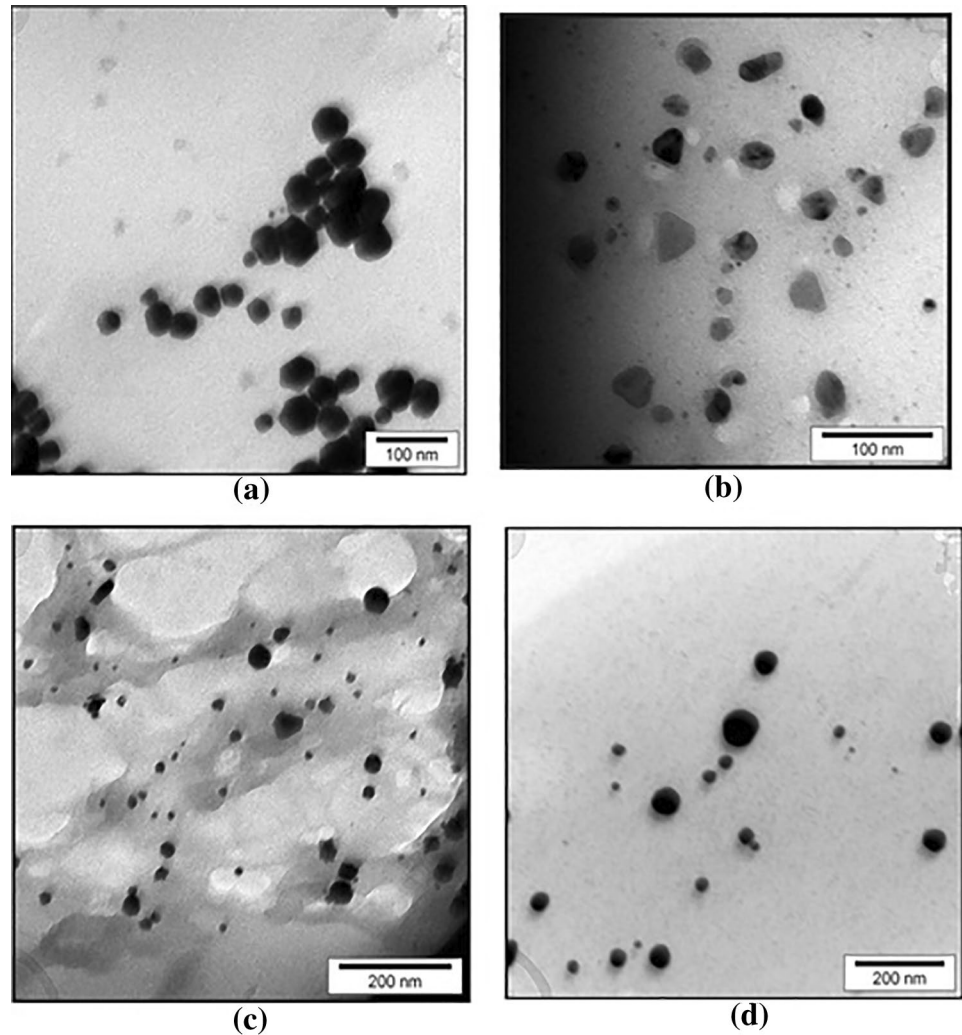
Row	Fungus	Wave-length (nm)
1	<i>Fusarium oxysporum</i>	530
2	<i>Sclerotinia sclerotium</i>	526
3	<i>Aspergillus flavus</i>	534
4	<i>Paecilomyces variotii</i>	539
5	<i>Colletotrichum gloeosporioides</i>	525
6	<i>Rhizoctonia solani</i>	520
7	<i>Verticillium dahliae</i>	534
8	<i>Peceaiomyces</i> sp.	537

of the fungus used in their study. As mentioned before, the difference between the species can also affect the size of the nanoparticles.

4 Conclusion

In this study, the capability of 12 fungi in the synthesis of gold nanoparticles was evaluated based on the color change observational method and UV–vis spectrometry. These evaluations of the fungi showed that out of 12 fungi scrutinized, 8 can synthesize gold nanoparticles. In the next stage, the fungi *Fusarium oxysporum*, *Aspergillus flavus*, *Rhizoctonia solani*, and *Verticillium dahlia* were selected for further investigation via transient electron

Fig. 4 The TEM micrographs for gold nanoparticles synthesized by the fungi **a** *Verticillium dahliae*, **b** *Fusarium oxysporum*, **c** *Aspergillus flavus*, **d** *Rhizoctonia solani*



microscope (TEM) and X-ray diffraction (XRD) analyses. The main results of this research can be summarized as follows:

- 1 In the UV–vis spectrometry, the maximum and minimum amounts of absorption belonged to the fungus *Rhizoctonia solani* and *Peceaiomyces* sp., respectively. According to the TEM micrographs, the average size of the nanoparticles for the fungi *Aspergillus flavus* and *Rhizoctonia solani* was about 30 nm. In addition, *Fusarium oxysporum* and *Verticillium dahlia* synthesized the nanoparticles with an average size of 20 and 40 nm, respectively. The synthesized nanoparticles for the examined fungi were intercellular with spherical, triangular and polygonal shapes. The maximum number of the synthesized nanoparticles belonged to the fungus *Rhizoctonia solani*.
- 2 Based on the XRD analysis, the synthesized particles are gold nanocrystals.
- 3 The species of the fungus affects the size of the synthesized nanoparticles.

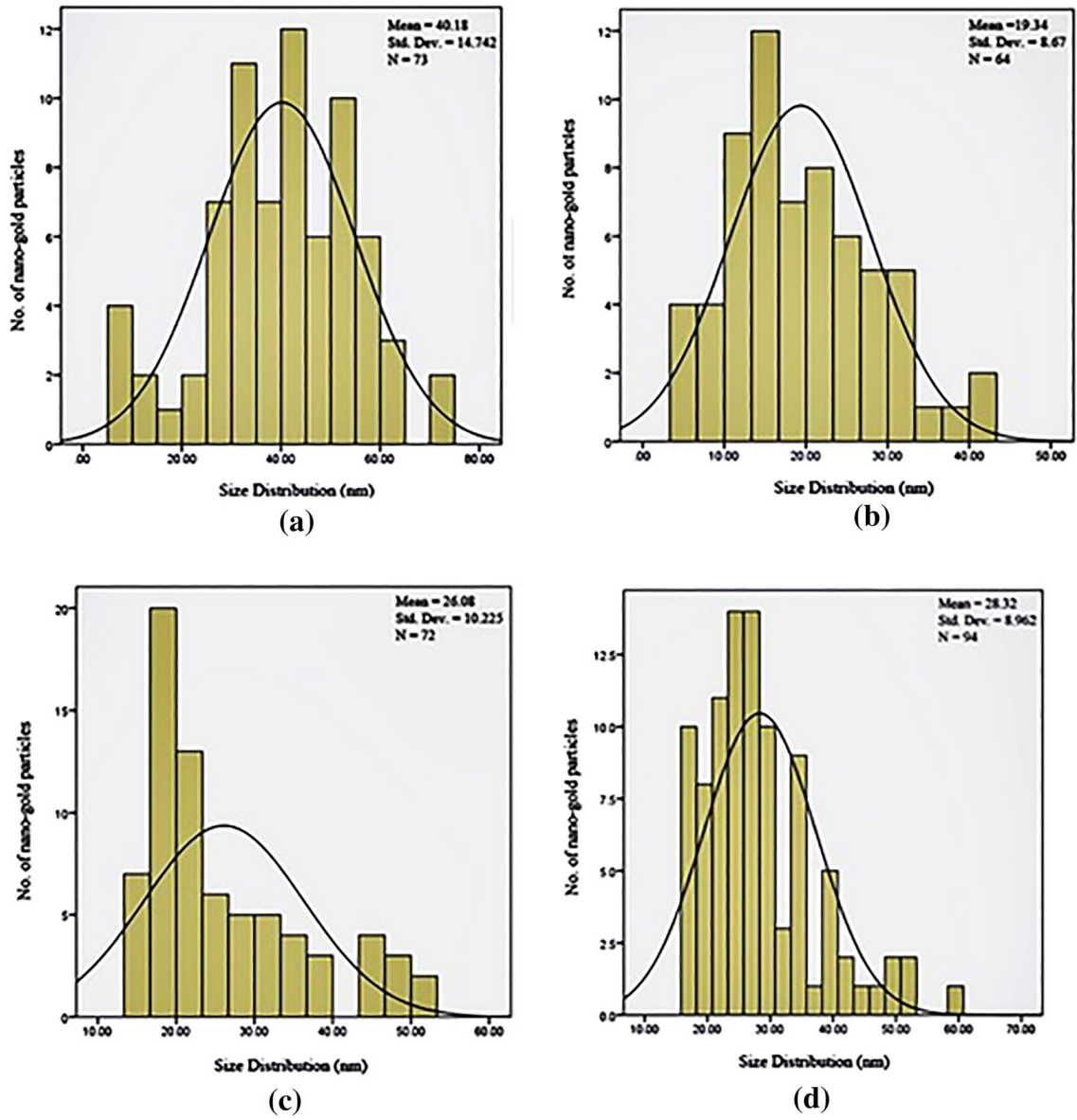


Fig. 5 Histogram of the size distribution of gold nanoparticles synthesized by the fungi **a** *Verticillium dahliae*, **b** *Fusarium oxysporum*, **c** *Aspergillus flavus*, **d** *Rhizoctonia solani*

Fig. 6 XRD pattern for gold nanoparticles synthesized by the fungi **a** *Aspergillus flavus*, **b** *Fusarium oxysporum*

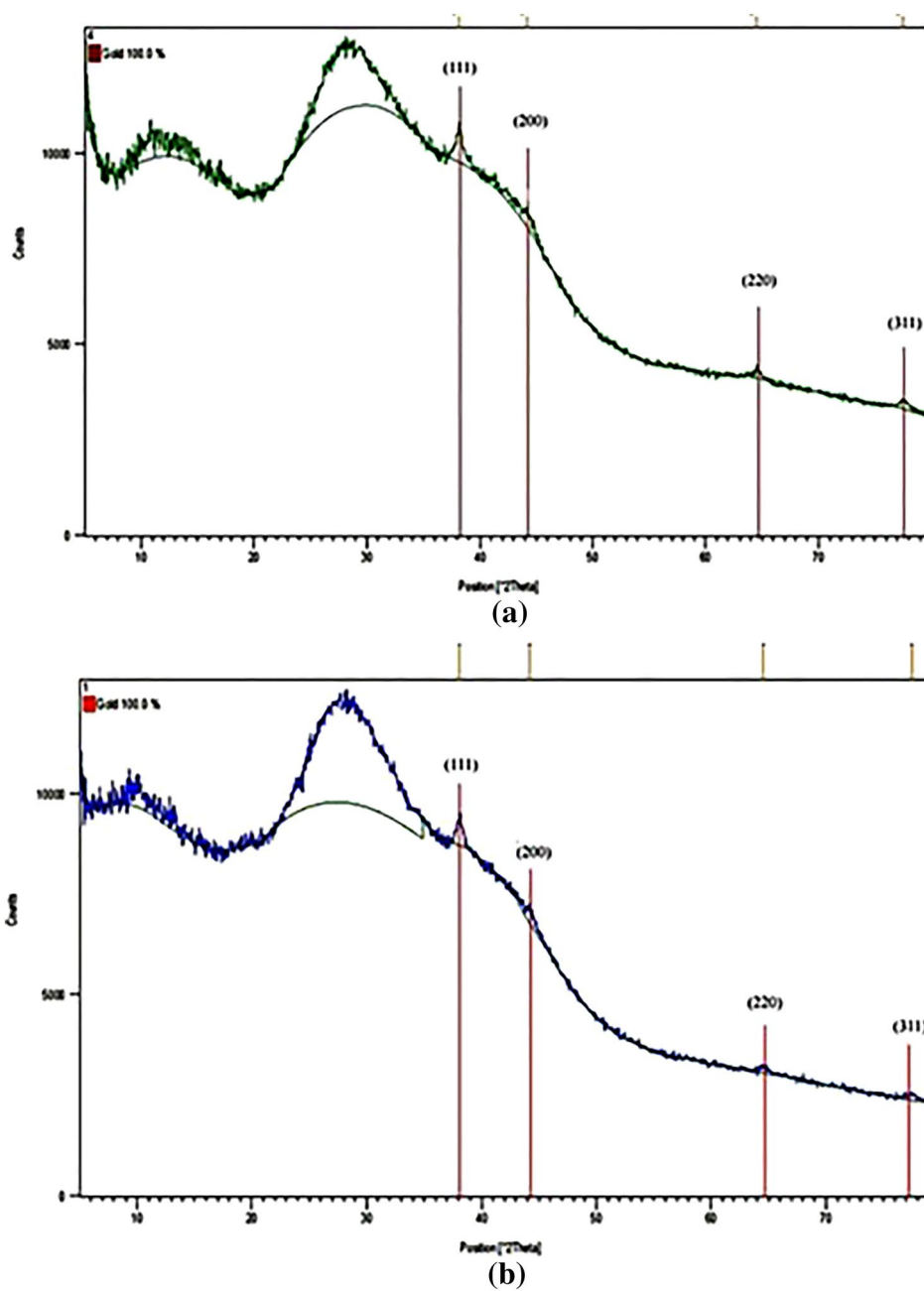
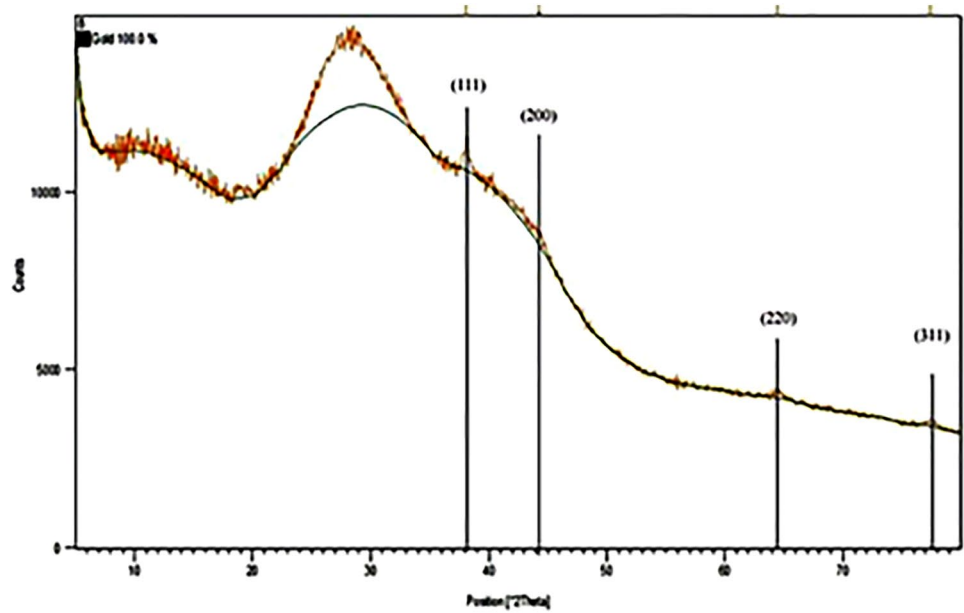
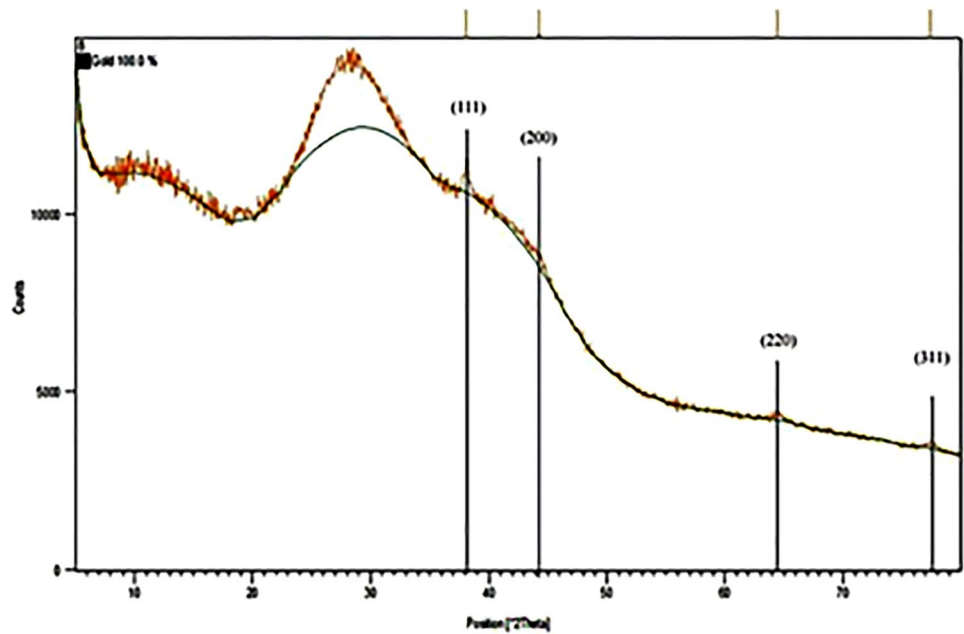


Fig. 7 XRD pattern for the gold nanoparticles synthesized by the fungi **a** *Rhizoctonia solani*, **b** *Verticillium dahliae*



(a)



(b)

Acknowledgements This research was financially supported by Iran National Science Foundation Grant No. 91003706.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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