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Article

Biosynthesis Nanoparticles and study the effect against some contaminated fungi

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ABSTRACT

This study aims to isolate some contaminated fungi (Aspergillus flavus, Arthroderma insingulare, Alternaria Alternata, Penicillium Chrysogenum, Penicillium expansum, Candida krusei, Candid famata) and treat them with nanoparticles synthesis from synephrine. The characterization of the prepared nanoparticles was done with Ultraviolet-visible spectroscopy, Atomic force microscopy (AFM), and Scanning electron microscopy(SEM). The most effect of NPs was observed in percent 1: 2, and the most affected fungus affected by NPs was Arthroderma when the diameter in control was (9.767) while in NPs was (5.233).

Keywords: Biosynthesis, Nanoparticles, contaminated fungi

INTRODUCTION

Since the development of nanotechnology, many approaches for nanoparticle synthesis have been discovered and improved [1]. The biological activity of AgNPs has been extensively studied in recent years; thus, their unique antibacterial, antifungal, and antiviral features have already been characterized in detail; at the same time, it was suggested that AgNPs might have a potential in cancer therapy owing to their prominent anti-proliferative and cytotoxic features [2]. Exposure effects to fungi are dependent on the species present, the metabolic products, concentration and duration of exposure, and also individual susceptibility [3].

MATERIALS AND METHODS

Fungi caused contamination

Fungi were isolated from the floors of 20 schools in Baghdad, Karkh, and they were collected randomly from November 2019 to February 2020. it was the most frequense Aspergillus flavus . The samples are put in transparent swaps, transformed into lab, and used for isolation and identification of fungi.

Biosynthesis of silver nanoparticles:

Silver nitrate (1x10-3) AgNO3 stock solution was prepared in sterile deionized triple-distilled water, and subsequent dilutions were made from this stock solution. This stock solution was prepared in the dark by adding (0.15) gm of AgNO3 in (1) L of DW. The DW was added to the aqueous extract solution with 100% concentration at a ratio (DW: extract) (3:1), then was added AgNO3 solution to the last solution at a ratio (AgNO3:solution)(1:2). Then, the last solution was exposed to sunlight for (7) days.

Characterization of the prepared nanoparticles

Ultraviolet-visible spectroscopy An ultraviolet-visible spectrophotometer (UV-Vis) refers to absorption spectroscopy. The samples were measured by UV-VISdouble beam spectrophotometers from 300-600 Wavelength.

Atomic force microscopy (AFM)

A thin film of the sample from each type of nanoparticle was placed on a glass slide by dropping 100 µl of the sample on the slide and was allowed to dry for 5 min. The slides were then scanned with the AFM [4]. C. Scanning electron microscopy (SEM) A small drop of each type of nanoparticle was placed on a carbon-coated copper grid and allowed to dry using the mercury lamp for 5 min. Then, readings were taken at a magnification of 5000x, 10000x, 20000x, 50000x and with steady voltage [5].

Estimation of silver nanporticles'anti-fungal activity.

AGAINST MOLDS:

1- We mixed the nano solution at a ratio(1:2) with the media at a ratio (1:10) (nano: media) and used the DW as a control.

2- We took pure colony by needle and culture in the middle petri dish.

3- We incubated at 37°C for 7 days

4- We studied the effect of silver nanoparticles on mold by measuring the diameter of the growth zone.

Against Yeast

1- We repared tubes with (5) ml DW

2- We took a swap from the colony by loop and got in this tube. 3- shake the tubes by vortex.

4- Take a touch from this tube and spread it on SDA media in a petri dish by loop.

5- Made pore in media and put 100 µl nano solution (1:2) in every pore by micropipette, and used the DW as control. 6- incubated this petri dish for 7 days.

RESULTS

When putting $AgNO_3$ solution with an aqueous extract of Synephrine as control, all flasks were exposed to slight sunlight at 26±2 °C for 7 days. The color change was observed, indicating the formation of AgNPs [6].

Characterization of AgNPs using changing color

The changing color of the mix (aqueous extract plus $AgNO_3$ solution) takes place from white white to light brown and dark brown color. In contrast, no color change is observed in the aqueous extract without $AgNO_3$ solution because of the role of Synephrine extract in the mix as a reductant and stabilizer agent. The change in color from white to light brown indicates the biosynthesis of AgNPs.

Results of color change indicated that Synephrine extract could be used as a reducing and stabilizing agent for the synthesis of AgNPs.

Characterization of AgNPs using UV-Visible Spectroscopy:

AgNPs were characterized by a UV-visible spectrophotometer. The UV-visible absorption spectra of the silver nanoparticles were measured in the range of (200-800) nm using a UV-visible spectrophotometer. UV-visible spectroscopy is an important and oriental technique for the characterization of nanoparticles. A strong and broad surface plasmon peak located at (230) nm was observed for the silver nanoparticles prepared by using extracts of Synephrine, as in Figure (1). The strong surface plasmon resonance centered at (230) nm indicates the formation of silver nanoparticles, which is very stable, with no evidence of accumulation of the particles even after one month. Silver nanoparticles synthesized from the aqueous extract of Synephrine after (7) days of exposure to sunlight. The formation of silver nanoparticles was confirmed by the UV-Vis spectrophotometry, which showed a strong peak within the range of (200-400) nm.

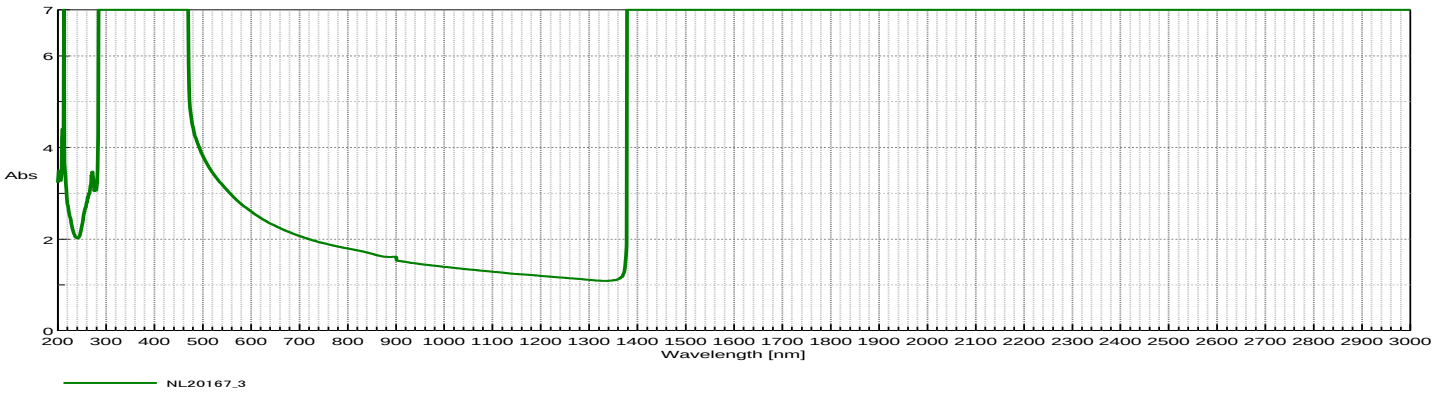

Figure 1. UV-Vis absorption spectra of silver nanoparticles after bio-reduction by the aqueous extract of Synephrine

Characterization of AgNPs using atomic force microscope (AFM) :

The results of AFM analysis showed both the two-dimensional and three-dimensional view of AgNPs; they were spherical, single or in aggregates. AFM analysis also showed that the average size of particles was 52.67 nm for AgNPs.

Characterization of AgNPs using Scanning electron microscopy (SEM)

The results of SEM analysis showed the particles are spherical (this is for 2 types of synthesized nanoparticles), with nanometer-sized AgNPs, figures (2). Large nanoparticles were seen due to aggregation. This aggregation took place due to the presence of cell components on the surface of nanoparticles and acts as a capping agent (Helen and Rani, 2015). This result agrees with Surega (2015), who observed the morphology of the synthesized AgNPs using aqueous extracts of Synephrine through SEM. The observations revealed that the AgNPs were spherical and agglomerated.

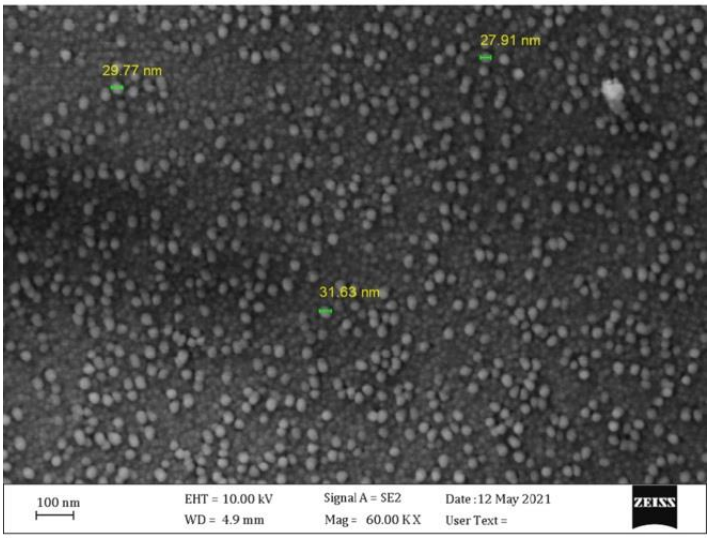

Figure 2. SEM image showed the shape and size of AgNP nanoparticles.

Estimation of anti-fungal activity for silver nanoparticles against fungi:

Fungus	Control	AgNps
Mold		
Aspergillus flavus	9.767	5.333
Arthroderma	9.767	5.233
Alternaria alternata	9.767	5.267
P.chrysogenum	9.767	5.267
P.expansum	9.767	5.300
Yeast		
Candida krusei	0.800	3.900
Candida famata	0.767	3.933

Table 1. Effect of silver nanoparticles of Synephrine against fungi

DISCUSSION

The formation of the color change is due to the excitation of surface plasmon vibration in metal nanoparticles, and the formation of AgNPs was confirmed by UV-Vis spectroscopy (Johnsona et al.,2014).

The previous study for synthesizing silver nanoparticles from Synephrine extract, SEM analysis, showed the average particle size of 20–30 nm and is spherical [7].

In the previous table, the effect of silver nanoparticles and the control on the inhibition of mold growth used in this study was observed. When the silver nanoparticles were applied to the samples, the diameter of the mold colonies was less than the diameter of the colonies over which control was applied. The table showed that Arthroderma had an inhibition effect on growth when treated with silver nanoparticles, which was more affected than other species. It was followed by P.chrysogenum and Alternaria alternata, which inhibited when treated with silver nanoparticles, which means the silver nanoparticles had a strong effect on this species by making less growth capability for this cell species. Aspergillus

flavus and P.expansum had close effects on each other. Each other had an inhibited effect when treated with silver nanoparticles; this material can be used as therapy for this isolated pathogenic fungi. Al-Zubaidi, Bocate, Al-Bahrani and Alananbeh agreed with this result. They demonstrated that A.flavus had inhibition when treated with silver nanoparticles [8]. While Candida krusei and Candida famata. The results of their response to silver nanoparticles were very close to each other; Candida krusei inhibited growth when treated by the silver nanoparticles, which means the silver nanoparticles affected the growth of this species, comparable to the control; the control did not affect this species. This result demonstrated that this species had an inhibitory response when treated with silver nanoparticles [9].

CONCLUSIONS

The diameter of the colony Aspergillus flavus was affected less when treated by nanoparticles comparable to the diameter of the same species but when treated by the control material. In yeast, the inhibition zone of Candida krusi forms when using NPs (3.900).

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